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# OBSERVATIONS ON THE BLOOD SUCKING ACTIVITIES OF THE HOOKWORM, *ANCYLOSTOMA CANINUM*

HERBERT S. WELLS

Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee.

## *Observations on the Blood Sucking Activities of the Hookworm,* Ancylostoma caninum [1]

The question as to whether or not hookworms suck blood has remained a matter of controversy for many years chiefly because observations on the worms have been carried out only after the death of the host. The observations to be described, which deal with the behavior of hookworms of the dog attached to the living host, show that these worms are indeed blood suckers.

Specimens of *Ancylostoma duodenale* removed from the host at autopsy have previously been observed to eject blood both from the mouth and the anus. Leichtenstern (1886) states that Grassi was the first to make such an observation. He states further that his own observations lead him to believe, with Grassi and Perroncito, that the parasite withdraws much more blood than is necessary for its food requirements, that the red cells are practically unchanged by passage through the worm, and that therefore the plasma must be the main source of nourishment. Ernst (1888) observed the ejection of blood from the mouth capsule as well, under similar conditions. Whipple (1909) saw these phenomena in Necator as well as in Ancylostoma and believed that they indicate a rapid ingestion of blood by the parasite. In spite of the fact that such observations indicate that the hookworm is a blood sucker, certain evidence has been adduced, mainly by Looss (1905) and by Ashford (1910), to indicate that blood is not the normal food of the parasite. In the first place these authors point to the fact that many worms seen at autopsy are empty of blood, even when they remain attached to the intestine. In the second place tissue elements are found in the worms, often consisting of long shreds of mucosa extending from the point of attachment of the worm far down into its intestine. From this it

1. A preliminary report covering the findings of the earlier experiments on this subject appeared in Science, *73*, 16, 1931.

has been concluded that the parasites subsist on the mucous membranes of the host, and that blood in the tract is merely evidence of accidental hemorrhage occurring during the process of biting into the tissues.

The views of Looss and of Ashford as regards the question of blood sucking, together with the evidence which has accumulated to indicate that the anemia of hookworm disease is not primarily due to loss of blood, have doubtless combined to discourage adequate investigation of these problems. From direct observations on some 200 worms involving the use of 20 dogs of various ages and degrees of infestation it is necessary to conclude that mucous membrane occurs in the worms incidentally to the need of the parasite for obtaining a constant and copious supply of blood, a conclusion which is opposed to the teachings of Looss and of Ashford. It is also clear, again in opposition to the views of these authors, that the presence of blood in the worms is anything but fortuitous and that in fact the supposition of Leichtenstern (1886) that the hookworm is a "luxurious consumer," [2] excessively wasteful of the blood of its host, is correct, at least in so far as the hookworm of the dog is concerned.

In the studies to be reported the worms were observed mainly while attached to the mucosa of the intestine of the living host, but certain details of the blood sucking process were found to be more easily studied by supplementary observations on isolated worms. Inasmuch as the methods employed involve the use of special apparatus as well as an operative technic not ordinarily applied in parasitological investigations it has seemed advisable to describe the procedures in some detail.

### *Preparation of the Dog for Observation of Worms Attached to the Intestine*

A dog is selected whose stool is positive for hookworm ova. A heavy infestation is not necessary, as all the worms present in the tract may be collected at the operation and applied to the particular segment of the gut selected for observation. Ether anesthesia may be used, but sodium barbital (soluble barbital, U. S. P. X.) is especially satisfactory because of the ease of administration. It gives a uniform deep anesthesia, requiring no special apparatus or attention, and lasting many hours. For this purpose 0.35 gram per kilo of body weight is injected intravenously in approximately 10 per cent solution in warm water. The vein situated on the lateral side of the dog's hind leg, just above the ankle joint, may be used for the injection. As this is a superficial

2. "Ja es scheint mir mit Grassi, 'mi venne il sospetto,' dass die Ankylostomen, wie ich es ausdrücken will, 'Luxus-Consumenten' der schlimmsten Art sind, indem sie wahrscheinlich weit mehr Blut entziehen und wieder per anum entleeren, als sie zu ihrer Ernährung nothwendig haben."

vein it is entirely unnecessary to use local anesthesia for the injection. In 15 minutes to 1 hour the animal will be completely anesthetized, as may be determined by pinching the skin of the abdomen with forceps. An incision about 4 inches long is made in the mid-line of the abdomen, and a loop of small intestine pulled out.

In order to hold the operated loop of intestine in a horizontal position and to check the contractions of the intestinal muscle, which will otherwise cause difficulty in keeping the worms in focus under the microscope, it is desirable to use a special supporting clamp of some kind. Such a clamp is pictured in Text figure 1. It is made from brass plates 5 mm. thick. The top piece is 4 cm. by 11 cm. and is cut out in the center to provide an ellipsoidal opening 2.5 cm. by 7.5 cm. Around the edge of this opening, on the lower side, there is a ridge about 3 mm. wide and 1 mm. high, which causes the greatest pressure to be exerted around the opening. The edges of this ridge are rounded so as not to cut the tissue. The ridge does not appear in the cut. The lower

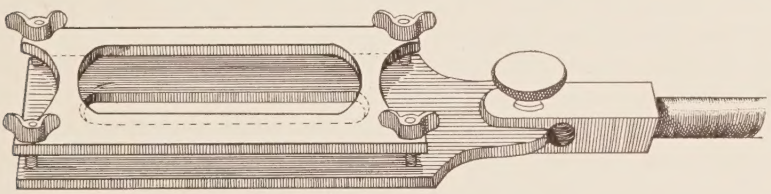

Fig. 1.—Intestinal clamp.

plate is slotted for the passage of the mesentery of the intestine, the slot being 9 mm. wide. The supporting rod, which is detachable, is 30 cm. long. The whole apparatus is nickel-plated.

The lower plate, separated from the rod, is placed over the loop of intestine and the loop pulled up through the hole in the plate. The mesentery is slit between the vessels at two places, leaving an intact blood supply to a loop of gut slightly longer than the plate. The ends of this loop are tied tightly with double ligatures of ¼ inch twilled cloth tape. The gut is cut through transversely between each pair of ligatures and the free ends are pulled back through the hole in the plate and replaced in the abdomen. The isolated loop is then opened longitudinally from end to end exactly opposite the line of attachment of the mesentery. As the incision is made bleeding is checked by applying spring paper clamps (obtainable at any stationery store) to the cut edges. The segment is then fixed in position by putting on the top piece of the clamp, which is tightly secured in place by the screws. The rod is then screwed to the plate and clamped to a heavy stand in such a way that the loop is supported horizontally just above the abdomen of the dog and without traction on the mesentery.

If worms are not present in the section of gut which has been opened it is necessary to tie off and remove other sections of the intestine, which may then be slit open and the worms pulled off by gentle traction with tissue forceps. The worms are then placed on the isolated loop where they will soon attach themselves to the mucosa. The chamber should be kept filled with warm isotonic saline solution. The dog's temperature, as recorded by rectal thermometer, should be maintained by means of a heating pad or other device. It is necessary, for illumination of the field under observation, to provide some type of microscope lamp provided with a condensing lens. A universal binocular microscope, of the type which is carried on a long arm that may be swung out over the dog, is best suited to the conditions of the experiments. A magnification of $\times 25$ is sufficient.

### *Technic of Observations on Isolated Worms*

Living hookworms have been observed previously under the microscope, usually immersed in water or saline. So far as I can determine, however, no one has observed their activity with blood as the surrounding medium. Such observations are easy to make and provide much information as to the mechanism of blood sucking.

A worm is removed from the mucosa by gentle traction with forceps and is placed on a slide. A drop of defibrinated dog's blood is then placed on the slide, and diluted with two or three times its volume of salt solution in order to render the preparation sufficiently transparent. A thick cover glass is placed on either side of the worm and a third coverslip mounted on top, thus imprisoning the worm in a narrow chamber and preventing too vigorous movements of its head. Examination is made with the ordinary low power objective, using transmitted light. It may be necessary to make several such preparations before a worm will be found which will suck blood.

### *General Description of the Blood Sucking Activity*

The actual process of blood sucking may be best observed as the worm first attaches itself to the mucosa, i. e. before the esophagus becomes buried too deeply between the villi. The process is especially easy to observe when the worm attaches itself to the tip or side of a villus. In the latter case one first observes that the vessels of the villus become congested and stand out very clearly as a bluish network. Even when the worm has become attached more deeply to the underlying tissues there are usually one or more such congested villi to be seen in close proximity to the point of attachment. The next thing to appear is a red disc on the surface of the villus, bounded by the edges of the attached mouth capsule. The mouth itself then fills with blood which

passes into the esophagus and thence into the intestine. The blood may be seen to be propelled by the sucking movements of the esophagus, which consist of the alternate expansion and contraction of its lumen, brought about by the action of the muscles of the esophageal wall. Sometimes a considerable amount of clear fluid, mucus and clumps of epithelial cells will be sucked in before blood is obtained, and this material may be also clearly seen as it is drawn into the esophagus and forced into the intestine. The supply of blood obtainable from the tip of the villus does not seem to be adequate, for the worm will soon release its hold and with vigorous boring movements work its head between the villi to a deeper point of attachment. The mouth capsule can then no longer be seen, but vigorous sucking movements of the esophagus can still be observed. As before, the first material taken in may not consist entirely of blood, but soon a copious supply of blood is tapped and the boring and twisting movements cease. As the body of the worm becomes quiet the esophageal movements become very rapid and forceful. The intestine of the worm fills rapidly with blood which soon dilates the anal end of the canal. There then occurs a forcible contraction of the muscles in this region, which often takes place with sufficient force to move the whole posterior part of the worm. A droplet of blood appears with great suddenness from the anal orifice.

The process may continue indefinitely, blood droplets being ejected frequently throughout the day. The blood so ejected does not appear to have undergone digestive changes. The whole process therefore presents certain features which are in marked contrast to our usual conception of the feeding habits of such parasites. It will therefore be desirable to consider in some detail the various aspects of this activity of the worms as brought out by the series of observations.

### *The Attachment of the Worm to the Mucosa of the Intestine*

When a worm is placed on the surface of the mucosa it will writhe about very vigorously, often traveling a considerable distance before attaching itself. Some worms, apparently more by chance than by intention, succeed almost at once in pushing their heads between the villi. The mouth capsule, assisted by rapid flexing movements of the cephalic portion of the body, is thrust into contact with an individual villus and the worm becomes attached. Other worms will move about for hours without attaching themselves. These worms may often be made to take hold more quickly by pushing their heads down into the mucosa by means of gentle pressure with a blunt instrument or gauze sponge. The success attending this procedure suggests that under normal con-

ditions the presence of folds and ridges in the intestinal surface may greatly facilitate the process of attachment.

A certain amount of force must be exerted by the body of the worm in order to thrust the mouth capsule into the tissues of the host. This force is brought to bear by movements of extension of the anterior body of the worm again the support provided by the posterior part of its body, which remains flexed and in contact with the mucosa. That these thrusting movements are essential for gaining a firm attachment is shown by the fact that the cephalic portion of the worm when separated from the rest of the body by transection just behind the esophagus still performs vigorous sucking movements and is able to twist about and move considerable distances in search of an attachment, but is unable to attach itself firmly. Attachment to the tip of a villus may occur and some blood may be obtained, but the head alone, vigorous as are its movements, is greatly handicapped mechanically in its obvious attempts to push deeper into the tissues and the attachment, in the absence of the thrusting movements of the body of the worm, is necessarily superficial and insecure.

One gains the impression that even under the best of conditions the parasite must perform a good deal of work before it becomes attached to the deeper tissues. Superficial attachment can occur in a few seconds, but it may be several hours before the worm has worked its way in to a depth corresponding to the usual condition of worms found when the intestine is first opened. Should the villi become swollen and edematous as the result of inflammation it may be impossible for the worms to become attached securely. Under these conditions they cannot get their heads down between the villi. The worms are unable to attach either to the freshly exposed surface of muscle tissue or to the mucous membranes of the tongue of the dog, although they will make vigorous attempts to do so. These tissues are apparently too tough to allow proper penetration of the mouth capsule and head. It would seem that the worms are not particularly well adapted for attaching to living tissue in general, and that were it not for the especially favorable conditions presented by the intestinal mucosa a firm attachment would be impossible.

### *The Process of Blood Sucking*

It would appear that the hookworm attaches to the intestine primarily for the purpose of obtaining blood. If a worm is placed on the intestine in a pool of blood it will often suck up this material without attaching to the mucosa. But the presence of soft, yielding tissue in contact with the head of the worm seems also to provide a part of the normal stimulus to blood sucking, for when a worm is submerged in defibrinated blood on a glass slide it will seldom suck blood at a rapid rate, while

if a small piece of mucosa is also placed on the slide, in contact with the head of the worm, the parasite will usually start to suck blood at once, and at a fairly rapid rate. The worm may attach loosely to this dead tissue, but seems satisfied not to penetrate deeply, as is necessary under normal conditions to reach the supply of blood. Substitution of coarser materials, such as cotton fibers, sponge or cloth, for the tissue in this experiment fails to elicit sucking movements. In the absence of tissue, the isolated worm may refuse to suck blood and will continue to throw its head restlessly from side to side for hours. Occasionally it may attempt to thrust its mouth capsule into the side of the glass coverslip. When an air bubble is present near the head of the worm, the parasite will repeatedly attempt to thrust its mouth into it, and will often begin to suck in the material—air as well as blood. The air seldom passes into the intestine, being usually regurgitated as soon as the esophagus becomes distended with the gas. The air bubble, by its surface tension effects, seems to act as a fair substitute for the intestinal villi in providing the stimulus of soft material necessary, in addition to the presence of blood, to provoke rapid and continued sucking movements.

Sucking may occur in the absence of a tissue stimulus in worms submerged in blood on the slide, but the sucking is seldom very rapid and is never continuous. When the worms are submerged in salt solution instead of blood, sucking does not seem to occur at all, for such worms have been watched for many hours under the microscope without any signs of esophageal movements being detected.

Worms are seen to suck blood up to the time of complete failure of the circulation of the host when the latter is killed in any manner. In fact, sucking movements are often seen to continue for many minutes after the death of the dog. Clear fluid, small amounts of dark venous blood or fragments of tissue may be aspirated by the vigorous sucking which occurs under these conditions. But sucking is not rapidly repeated. The esophageal movements are infrequent and the activity of the worm seems mainly directed either toward finding a deeper attachment in the same place or to a search for a new position. On one occasion, where 15 worms were being observed, the dog, which suffered from a high degree of anemia, died very suddenly. Within 10 minutes all worms had left their former places of attachment and many had taken hold in fresh locations. In the case of several worms each was seen to attach itself in five or six spots during the hour of observation following the death of the dog. Some worms remain attached indefinitely after the death of the host. The heads of these worms are usually so deeply embedded in the tissues that the esophagus cannot be seen, but in cases where observation is possible, it is seen that these worms are lying quietly, making only feeble and occasional attempts

to aspirate from the tissues. The inactivity of worms on the dead host presents, in general, a striking contrast to the conditions observed during the life of the host, and indicates that the presence of soft tissue alone is not a sufficient stimulus to provoke normal sucking movements.

As the body of the dog becomes cold after death all worms become less and less active until finally they do not move at all unless a special stimulus is applied. Such a stimulus is heat. Warm saline will cause the worms to become quite active. This fact was noted by Leichtenstern (1887) who removed worms at autopsy which were apparently dead but which could be revived by warming them. But the general activity of bodily movements of a worm tells us nothing of the activity of sucking movements. Isolated worms at temperatures from 20° C to 25° C, although they are relatively inactive with respect to general bodily movements, will nevertheless suck blood. Consequently it is not the cooling of the worms after the death of the host that causes sucking movements to cease but rather the absence of the supply of blood. Such cooling alone will slow the rate of sucking movements but will not abolish them.

In the case of worms attached to the living dog, where the optimum conditions for blood sucking exist, it is noted that the rate of the sucking movements of the esophagus is usually very rapid, rates from 120 to 250 per minute being not unusual. The temperature of the salt solution bathing the worms was usually from 30° C to 35° C. When, as frequently happens at variable intervals, the blood supply appears to become inadequate, the rate is slower and there is no special rhythm, except that there may be regular runs of 10 to 20 pulsations, followed by intervals without pulses or with only an occasional pulsation, during which intervals the worm twists about, attempting to thrust its head deeper into the tissues. The cause of these apparently spontaneous failures of the blood supply is not clear. It is possible that they may be the result of coagulation of blood in the small eroded vessels.

Although sucking occurs almost continuously in the majority of worms, occasionally a worm will be found which will lie quietly for several minutes without making any attempt to suck blood and without trying to burrow deeper into the tissues. In most of the experiments such inactive periods occurred very rarely and were of short duration. But in 4 out of the series of 20 dogs used, practically all the worms observed on each host were obviously much less active as regards blood sucking and blood ejection than the majority of worms in the other hosts of the series, and periods of complete inactivity were frequent and prolonged. There would therefore seem to be two types of inactivity as regards blood sucking: the first type, as it is of a temporary nature and affects different worms at different times, is most probably referable

to factors inherent in the worm; while the second type, being prolonged and affecting all or practically all of the worms of a given host, is probably to be referred to environmental influences.

No attempt has been made to determine the cause of the first type of inactivity, which may represent merely periods of physiological rest for recovery from fatigue. But as regards the second type it seemed desirable to try the effects of as many kinds of changes in the environment as possible in order to discover what factors external to the parasite can influence blood sucking. It was hoped that some evidence might be forthcoming which would indicate whether the conditions of the experiments were in general favorable or unfavorable to this process so that the probable degree of activity of the worms under strictly normal conditions might more readily be estimated. The external environment of the worm consists on the one hand of the blood and tissues of its host and on the other hand of the medium in which its own body lies, viz. the contents of the intestine of the host. Host variations may be found in the age, sex, degree of infestation, depth or type of anesthesia induced, body temperature, time elapsed since the last meal, type of diet, composition of the blood, etc. Variations produced in the surrounding medium may involve changes in such factors as the intensity of the light, the temperature, the reaction, and the chemical composition of the solution including the tension of dissolved gases. With the exception of changes in these various factors which lie outside the possible physiological range, such as temperatures below 25° C and above 40° C and light of very high intensity, variations in these factors, either as provided by the random choice of dogs or as produced by experimental procedures have so far failed to show any correlation with the degree of blood sucking activity. For example, nothing has been found which will stimulate inactive worms to suck blood more rapidly, and conversely no procedure designed to reduce or abolish the activity of the more vigorous blood suckers has been successful. Active worms will continue to suck blood vigorously up to the point of complete circulatory failure as the dog is asphyxiated or is more deeply anesthetized with ether or chloroform. They will suck actively when transferred to the intestine of an anesthetized rabbit. Their activity is not affected by the complete absence of anesthesia in the dog, as was determined by placing several worms on an exteriorized loop of jejunum in a previously operated animal. In this case a jejunal fistula allowed part of the normal duodenal contents to flow out onto the exteriorized mucosa, so that the worms could be studied in as nearly normal an environment as possible. The results of this experiment seem to justify the assumption that barbital anesthesia of the host does not affect the blood sucking activity of the worms, and the experiment therefore serves as a control, as regards the

effects of this anesthesia, for the whole series of anesthetized dogs studied. It seems worth mentioning that even during copulation both the male and the female worms are usually attached to the mucosa and are sucking blood rapidly. In fact as much blood has been seen to be ejected from the cloaca of the male under these circumstances as in the free condition of the parasite, the blood escaping from beneath the folds of the bursa and passing out into the surrounding salt solution. An even more surprising phenomenon is that of the continuation of blood sucking, with no diminution in vigor, when the worm is completely transected just behind the esophagus. The head pumps blood as actively as before. When it is pulled away from its attachment it will twist about as best it can, the mouth capsule being moved in so effective a manner that a new attachment may be achieved. In one instance enough blood was obtained to fill the esophagus, and the head persisted for over an hour in its efforts to reach a more abundant source of blood.

In general one may conclude that the worms are remarkably resistant to changes which might be expected to influence the rate of blood sucking. Consequently there is no reason to suppose that the active blood sucking observed in the majority of cases can represent an abnormal behavior of the worms, attributable to an influence of extraneous factors introduced in the operative procedures employed.

The details of the process of sucking may be studied best under the microscope, which allows the movements of the esophagus to be analyzed. The esophagus dilates, apparently through the action of the intrinsic musculature of its wall, thus filling with blood, which ordinarily flows in only from the mouth capsule. The posterior end of the esophagus at the same time closes, preventing regurgitation of blood from the intestine. This closure is apparently effected by the esophago-intestinal valve; at least the lumen may be clearly seen to be closed only at the narrowest point, corresponding to the position of this valve as described by Looss. As the walls of the esophagus collapse the esophago-intestinal valve opens, allowing blood to be forced into the intestine. But this single valve mechanism does not suffice, unaided, to determine movement of the blood into the intestine. Careful observation will demonstrate that as the esophagus contracts its anterior end becomes completely closed, preventing escape of blood into the mouth capsule. This closure occurs in the form of a narrow constriction of the lumen just back of the esophageal funnel.

That the worm may exert a certain amount of control over the working of these two valve mechanisms is evident from the fact that the movements of the esophagus may cause blood to pass entirely inward, without any regurgitation whatever; or blood may be entirely regurgitated, to the point of practically complete emptying of the intestine through the mouth; or a to-and-fro motion of fluid may occur, with

movement either predominantly inward or outward, but with considerable leakage at either or both of the "valves."[3] It is this ability of the worm to regulate the valve action of the esophageal pump which seems to explain why, when a number of worms are seen attached to the mucosa, the esophagus of each filling and emptying in rapid pulsating movements, some of the worms may be passing blood per anum at a rapid rate while others may for a considerable time pass little or no blood. In the one case esophageal pulsation produces ingestion of blood, in the other case the motion of blood is largely to-and-fro with regurgitation from the intestine at each filling of the organ.

In worms attached to the mucosa, one can often see fragments of epithelial detritus shining through the intestinal wall in the form of white masses. Such easily visible and freely movable particles serve to mark the direction and extent of movement of fluid in the intestine. They may often be seen to move rapidly to and fro synchronously with the esophageal pulsations. In general such movements carry the particles farther in the caudal direction than cephalad, so that the intestinal contents gradually move back, with a jerky motion. This type of motion may be suddenly interrupted by a continuous, rapid, caudad streaming, corresponding in time, obviously, to a more nearly perfect action of the valve mechanism. It has not as yet been possible to determine what factors influence the action of the valves so as to produce first one and then the other kind of motion of blood through the intestine.

That the force of the esophageal contractions producing the movement of blood is considerable and that the valve action may at times be very efficient is evidenced by the fact that relatively large pieces of mucous membrane can be passed through the intestine of the worm and ejected rapidly through the relatively narrow lumen of the rectum.

### *The Passage of Blood Through the Intestine of the Parasite*

In general there is blood in the worm almost constantly as long as the usual blood supply is available. This is particularly true of the females. In the male worm the tract is straighter, shorter and apparently holds less blood. Therefore, the reservoir being smaller, changes in the volume rate of blood sucking are more quickly reflected in changes of the rate of ejection. Thus, when blood sucking ceases for a few moments the tract of the male may quickly empty itself. The intestine of the female, on the other hand, usually contains some blood at all times, although with cessation of blood sucking the cephalad

3. I am informed that the technical term "valve" cannot be accurately applied to a valve-mechanism unless the structure of such mechanism corresponds to certain well defined morphological types such as have been especially studied in the nematodes. My use of the term "valve" must therefore be understood to refer to the function of the part rather than to the details of its structure.

portion of the worm may become colorless as blood passes backward and out through the anus. This does not indicate that the intestine itself is capable of passing blood backward by peristalsis but rather that the worm, in seeking for a new blood supply, is for the moment sucking in clear fluid or tissue elements, which displace some of the blood in the intestine.

When the worm migrates to a new point of attachment, when the host dies, or when the worm is removed from the living host, the intestinal tract of the parasite may or may not become quickly emptied, either by ejection through the anus or by regurgitation through the mouth. Some individuals become colorless very quickly under these conditions, while others may be kept alive for as long as 72 hours in saline solution without losing their blood. As movement of blood into and out of the intestine depends primarily on sucking or regurgitating movements of the esophagus and since many worms are found which, when detached from the mucosa, will not make any esophageal movements whatever, it is obvious that these will retain their blood. It may be concluded that it is due to individual differences in behavior of worms in this respect that determines the presence or absence of blood in their tracts on examination at autopsy of the host. The failure to find blood in a worm under such conditions obviously does not, as Looss and others have believed, preclude the possibility that the worm has been sucking blood in the recent past.

In observations on worms attached to the mucosa the blood ejected per anum is at times seen to be diffused freely through the saline solution bathing the parasites. At other times the material is more viscous and sticks to the worm or to the mucous membrane. In such cases, during the course of the day, the whole mucous surface, in a circle whose center is the point of attachment of the worm, will become coated with sticky masses of blood. The viscous material is not entirely blood, for at times it may be ejected in stringy masses which are only slightly blood-tinged. Whether this material represents mucus sucked up from the epithelium of the host or is a secretion formed by the parasite has not been determined.

The frequency of the ejection of droplets varies considerably, intervals between emissions varying from a fraction of a second to 15 minutes or more. Periods of great activity are followed by periods of comparative inactivity. During active periods drops may appear quite regularly at intervals averaging less than a minute, as illustrated in Table I. It is obvious that, in general, variations in rate of emission of blood will depend ultimately on variations in the rate of blood sucking.

For the purpose of measuring the amount of blood ejected it is necessary to collect an individual droplet in a capillary pipette, a procedure which is readily carried out. Into such a pipette, filled with

saline solution and provided with a rubber nipple, the caudal portion of a worm may be drawn with gentle suction. When a drop of blood is emitted the pipette is withdrawn and the ejected material made up to a known volume of 0.5 to 1.0 cc. The fluid is mixed and a drop placed in the chamber of a hemocytometer. With the high power objective the red corpuscles may be identified and counted. Calculation gives the total number of corpuscles emitted in one drop of blood. Knowing the red cell count of the dog one can calculate the size of the droplet in terms of the dog's blood. As determined in this manner, the size of droplets is found to vary from 0.05 to 0.50 cu. mm. One gains the very definite impression that drops collected in a pipette are, if anything, smaller than those normally emitted by the worm. It would certainly seem fair to assume that the average droplet consists of 0.25 cu. mm. of blood in the case of active worms. If one could assume that the ejection of droplets of such size would continue at the rate

TABLE 1.—*Observations on the Ejection of Blood Droplets from the Anus of a Female Worm*

| Intervals Between Successive Ejections, in Seconds | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55, | 10, | 40, | 15, | 45, | 10, | 15, | 15, | 25, | 18, | 32, | 40, | 15, | 35, | 15, | 15, | 12 |
| 43, | 10, | 20, | 15, | 35, | 30, | 10, | 30, | 30, | 15, | 30, | 30, | 40, | 15, | 50, | 10, | 35 |
| 60, | 15, | 30, | 20, | 15, | 45, | 35, | 20, | 30, | 15, | 20, | 15, | 50, | 48, | 2, | 2, | 38 |

Mean interval .....26 seconds  Number of drops..... 52
Duration of observation..22 minutes  Average drops per minute 2⅓

indicated in Table I, one would be justified in concluding that, in 24 hours, each worm would remove from the host 0.84 cubic centimeters of blood. As several hundred worms may be found in a single dog, it would appear that blood sucking may alone account for the anemia often found in such dogs, provided our assumption of practically continuous blood sucking at this surprisingly rapid rate is justifiable.

The color of the blood emitted is nearly always bright red, although occasionally it may be bluish. Hence, it may be assumed that the parasite is usually successful in tapping a supply of arterial blood. During rapid ingestion and emission no change in color of the blood from red to blue may be noted as the blood passes through the worm. However, when the passage of blood is less rapid, the blood at the caudal end of the worm may be distinctly blue as contrasted with that in the anterior end of the intestine and in the esophagus.

### *The Physiological Significance of Blood Sucking*

Although there can scarcely remain any doubt that the hookworm of the dog sucks blood under normal conditions, the reason for this activity of the parasite is not at all clear. It would appear that blood

passes through the worm at such a rapid rate that there can be no time for digestion of the material, and that consequently the worm must utilize simple diffusible substances already prepared for consumption by its host. Whether these substances include mainly oxygen for combustion or sugars and other substances for the fuel of metabolism has not been determined. One can only suppose that the thin-walled intestine of the worm serves, functionally, as a capillary blood vessel and that the esophageal pump acts as a heart.

That the intestine is permeable to oxygen is demonstrated by the changes in color of the blood passing through it. One is tempted to assume, therefore, that the whole process of blood sucking subserves mainly a respiratory function. The main difficulty in accepting such an hypothesis comes from the fact that the cuticula of the worm is also permeable to oxygen. When a worm is kept in blood under coverslips for some time the blood on either side of the parasite gradually becomes blue in color, showing that oxygen is able to pass out of the blood and into the worm. Furthermore, worms placed in a beaker of salt solution at room temperature become very sluggish in a few minutes if the beaker is filled with solution, while if only enough solution is used to just cover the worms or if a slow stream of air or oxygen is bubbled through the deeper solution, the worms remain lively for hours. Finally, when a stream of oxygen is allowed to pass over the mucosa of the intestine to which worms are attached, the blood inside the worms is seen to take on a brilliant scarlet color in a few seconds. In spite of the ease with which oxygen passes through the cuticle the blood sucking activity of the worms exposed to an atmosphere of the pure gas is not diminished, as much blood passing through the intestine of the worm as usual. This indicates either that blood sucking has nothing to do with respiration, or that the physiological mechanisms for adapting the degree of blood sucking activity to the oxygen need are poorly developed. In any case, whatever may be the relation between blood sucking and oxygen need, the oxygen consumption of the worms should be quantitatively studied. For, unless it can be shown that the relatively low oxygen tension of the dog's intestine can supply enough oxygen by cuticular diffusion for optimum activity of the parasites, it will be necessary to assume that the ingested blood supplies at least a part of the oxygen for the normal processes of metabolism.

Whatever the parasite may be able to utilize from the ingested blood, it would seem that blood is necessary to maintain the normal strength and activity of certain of the vital processes. For, whereas a worm recently removed from the host will, on replacing it on the intestine, suck blood immediately, a worm that has been kept for several hours in

salt solution either at 20° C or at 38° C will be very sluggish and may not at first be able to attach firmly to the intestine. If it is active enough to obtain even a small amount of blood, however, it will recover quickly, and in half an hour may be firmly attached, sucking blood vigorously, and appearing normal in every way. Egg laying, which usually ceases soon after removal of the worm from the host, was seen to be resumed, in the single observation made on this point, as soon as effective blood sucking had been established on replacing the worm on the host.

### *Relation of the Findings to the Question of the Causation of Anemia in Hookworm Disease*

One should be cautious in coming to any conclusion, on the basis of observations on *Ancylostoma caninum,* as to whether *Ancylostoma duodenale* or *Necator americanus* are blood suckers. Nevertheless it would seem very likely that an examination of the Old World hookworm, which is so like *Ancylostoma caninum* in structure and life history, would show this parasite to be a blood sucker. It would not seem so easy to prophesy the outcome of observations on the Necator in regard to blood sucking, for this worm differs from *Ancylostoma caninum* in several important respects. These questions might be answered in a fairly satisfactory manner by removing the live worms at autopsy on human cases and placing them on the intestine of the dog or other animal. If they suck blood under these conditions it might be presumed that they also suck blood from man. In this connection one may recall the observation described above which shows that the dog hookworm will suck blood when placed on the intestine of a rabbit. The question as to whether hookworms can suck enough blood to cause the anemia of hookworm disease is more difficult to answer, even as regards this disease in dogs. Where patients are concerned, one is confronted at once with the almost unanimous opinion of those who have done the most work with such patients that, since the stools of persons suffering from hookworm disease even in its most severe form are not highly colored, they cannot contain much blood. The assumption has been that in the presence of an appreciable amount of blood the stools will necessarily be colored. However, as there is no method by which the amount of blood in the stools may be quantitated, it would appear that this assumption has not been subjected to experimental tests. A quantitative method for the determination of blood should certainly be devised, if possible, and applied to the study of the amount of blood lost by hookworm patients. As suggested by Leichtenstern (1886) the question of the relation of loss of blood to the anemia might be settled by analysis of the stools for iron under properly controlled dietary conditions. So far as the author is aware, this has never been done.

SUMMARY

1. Observation of over 200 specimens of *Ancylostoma caninum* attached to the intestine of anesthetized dogs shows that under these conditions the worms are almost constantly sucking blood.

2. The process of attachment of the worms to the mucosa, the details of the blood sucking process, the passage of blood through the intestine of the worm and its ejection through the anal orifice have been studied. These studies have been supplemented by the observation of isolated worms immersed in blood.

3. The frequency of ejection of blood droplets and the volume of blood ejected in each drop have been determined in several instances. Calculation from such data indicates the possibility that a single worm may withdraw blood from the host at the rate of 0.8 cc. in 24 hours.

4. The ejected material appears to consist mainly of red corpuscles of the host, together with some epithelial material and bacteria.

5. From the rapidity of flow of blood through the worm it would appear that the parasite can utilize only simple diffusible substances already prepared for its consumption by the host.

6. The findings indicate that in any consideration of the causation of anemia of hookworm disease, whether in dogs or in man, the factor of blood sucking must be taken into account.

I wish to take this opportunity to thank Dr. J. W. Beard for performing the operation which made it possible to study the activity of the worms on an unanesthetized dog.

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# A NEW CESTODE FROM *RANA CLAMITANS* LATR.*

CARMEN PHYLLIS OSLER

The presence of tapeworms in frogs is unusual. Frogs of the species *Rana clamitans* Latr. taken from roadside ditches running into Burt Lake and Fontinalis Run on Burt Lake, Cheboygan County, Michigan, were found infested with tapeworms of the family Proteocephalidae La Rue 1911. These worms were studied by the writer, while at the University of Michigan Biological Station, the summer of 1930.

*Ophiotaenia saphena* nov. spec. is usually distinguishable before opening the small intestine of the host because of the transparency of the intestinal wall. When removed from the intestine of the host and placed in tap water, normal salt solution, or Ringer's solution modified for amphibians, the worms were very mobile. The scolex displayed true Proteocephalid characteristics, that is, a constantly changing form. Before killing, the cestodes were quite translucent, the single dead specimen was stained brown. The longest specimen measured 280 mm. in length, the two shortest specimens, 40 mm., the average of the fourteen specimens being 152.7 mm. The length was taken after the worms were killed with hot aqueous solution of corrosive sublimate and straightened out upon a glass plate.

Strobilation is absent in the long, slender neck region which composes from five to seven per cent of the total length of the worm. It is faint in the young and mature proglottids because the lateral margins are smooth and the transverse intersegmental furrows are very shallow. It becomes faintly evident to the naked eye in the gravid proglottids. The neck measures 4.17 to 5.25 mm. long and the minimum width ranges from 0.16 to 0.25 mm. The first young proglottids are about seven times broader than long (Fig. 3). Their lengths vary from 0.05 to 0.10 mm., their widths from 0.50 to 0.57 mm. The proglottids gradually become more quadrate until the mature proglottids have a length of 0.65 to 1.08 mm., the average being 0.93 mm., and a breadth of 0.57 to 1.0 mm., the average being 0.86 mm. Averages were obtained from ten measurements in each instance. Gravid proglottids enlarge to 0.83 to 1.34 mm. in length, average 1.08 mm., and 1.00 to 1.59 mm. in width, average 1.4 mm. The ruptured proglottids, those in which there is a ventral, longitudinal splitting of the uterus, remain attached for some time. From 17 to 35 of these proglottids remain attached

* Contribution from the Biological Station and the Department of Zoology, University of Michigan.

and because they have elongated considerably they make up about half of the total length. They measure 0.95 to 2.34 mm. long by 0.78 to 1.42 mm. broad, the average being 1.63 mm. by 1.1 mm.

Scolx ovoidal, spineless, 0.20 to 0.25 mm. long by 0.27 to 0.32 mm. broad. Four suckers prominent, globular, cup-like with entire margins, 0.12 to 0.15 mm. in diameter. A degenerate fifth sucker is present. In a frontal section of the adult scolex (Fig. 1), it is seen as an elongated, sac-like organ surrounded by a definite basement membrane and filled with a mass of nuclei and scattered fibers. It measures 49$\mu$ long by 23$\mu$ maximum width. A small tubular cavity lined with cuticula connects with the exterior and may be seen as a faint circle in a toto mount of the scolex (Fig. 3). It may be easily overlooked. This sucker is not as degenerate as that of *O. filaroides* La Rue (1909: 25) which consists of a submerged mass of tissue with no external connection, but is more like that of *Crepidobothrium gerrardii* La Rue (1914: 253) which retains its external connection. *P. tigrinus* Woodland (1925: 371) has an organ in the scolex apparently resembling that in the present species, but in the latter atrophy has proceeded somewhat further.

Genital pores marginal, irregularly alternating, located at the ends of the anterior third of the proglottids. Each pore has a slight papilla and communicates with a small genital sinus. The testes (Fig. 9) lie in the two lateral fields between the vitellaria and the median line, and dorsal to the uterus. There are 88 to 120 testes in the mature proglottids and they are almost spherical, measuring 0.05 to 0.08 mm. The vas deferens appears as a tube with 12 to 16 coils inflated with spermatozoa and extends from mid-field to the cirrus-pouch (Fig. 9). This mass of tubules continues into the cirrus-pouch as the ductus ejaculatorius. The protruded cirrus is an aspinose organ, 0.25 to 0.33 mm. long, with a blunt end, and a somewhat bulbous portion, 0.05 to 0.08 mm. in diameter adjacent to the genital pore. Frequently there are a few small coils of ductus ejaculatorius in the bulbous portion of the extruded cirrus (Fig. 2), but this cirrus with its small bulb and few coils of ductus ejaculatorius is far different from the short thick cirrus of *O. trimeresuri* containing many coils of ductus ejaculatorius. The protruded cirrus of this species is smaller than that of *O. marenzelleri* and has fewer coils of ductus ejaculatorius. The cirrus-pouch is a slightly muscular sac-like organ, 0.12 to 0.17 mm. long by 0.04 to 0.08 mm. broad. The ratio of the length of the cirrus-pouch to the breadth of the proglottid is 1:6 (Fig. 9).

Vaginal pore usually anterior to that of the cirrus-pouch; occasionally ventral or posterior. In the latter instances, the vagina crosses ventrally from the opening to its usual position anterior to the cirrus-

pouch. It extends almost straight from the pore to mid-field, then turns sharply toward the ovary (Fig. 9). It is dorsal to the uterus. Vagina larger in mature proglottids when it is irregularly distended with spermatozoa (Fig. 9), while in the ruptured proglottids it appears as a tube of uniform size. A slight enlargement occurs in the region dorsal to the bridge of the ovary which La Rue (1909:31) calls a receptaculum seminis, and then the lower vagina continues as a smaller, more uniform tube to the oviduct. The ovary extends across the posterior end of the proglottid between the vitellaria, and consists of lateral, wing-like lobes connected by a mid-piece (Fig. 9). The lateral lobes of the ovary are intermediate between the long, wing-like structures of *O. filaroides* La Rue (1909:33) and the long, straight, heavy bodies of *O. cryptobranchi* La Rue (1914:15). The oviduct (Fig. 8) arises from the small, globular oöcapt. In its course in the oötype, the oviduct curves to one side, then toward the mid-field where it unites with the vagina. The oviduct continues as the fertilization passage across the median line and, almost immediately after receiving the common vitelline duct, it curves sharply anteriorad, and enlarges into the oötype. The latter (Fig. 7) is an elongated organ with a heavy muscular wall, surrounded by the club-shaped epithelial cells or Mehlis' gland. From the oötype, the uterine passage proceeds anteriad and ventrad to the ovarian bridge (Fig. 8). No cilia were observed in the tubules of the female organs.

The uterus in gravid proglottids occupies one-half to two-thirds of the field between the vitellaria and extends the full length of the proglottid anterior to the ovary (Fig. 10). The 14 to 18 lateral pouches on each side may be either simple of bifurcated. The uterus ruptures in the median line at two points by ventral outpocketings as described by La Rue (1909:33). The first rupture usually occurs at the level of the genital pore and the second in the center of the proglottid (Fig. 10), and these are followed by the longitudinal splitting of the uterus.

The follicular vitellaria occupy the spaces between the lateral nerve-cord and the ventral excretory tube. The vitelline ducts pass from the vitellaria ventral to the ovary and unite in the interovarial space to form the common vitelline duct whose union with the oviduct has already been noted (Fig. 8).

La Rue (1911) founded the genus Ophiotaenia to include certain Proteocephalidae found in amphibians and reptiles. He recorded two species that had been found in the Amphibia of North America, *O. filaroides* inhabiting the intestine and rectum of *Ambystoma tigrinum* and *O. lönnbergii* found in the intestine of *Necturus maculosus*. An Australian form, *O. hylae*, was described by Johnston (1913) from *Hyla aurea*. La Rue (1914) described *O. cryptobranchi* found in the

intestine of *Cryptobranchus allegheniensis*. Hungerbühler (1910) described *Ichthyotaenia schultzei* from *Rana adspersa* in South Africa, and Woodland (1925) described *Proteocephalus tigrinus* from the intestine of *Rana tigrina* in India. *O. magna* found in the intestine of *Rana catesbeiana* was described by Hannum (1925). *Ophiotaenia saphena* adds another cestode to a rather short list of species of Ophiotaenia parasitic in amphibians. It resembles most closely *O. filaroides* in the general arrangement of the internal organs and in possessing a vestigial fifth sucker; however, the latter has not atrophied as much as that of *O. filaroides* and is more like that of *C. gerrardii*. In size, *O. saphena* is comparatively small, averaging about the same length as *O. lönnbergii*, somewhat larger than *O. hylae*, and much smaller than *O. magna*. It resembles *O. cryptobranchi* in that the vagina and vas deferens extend directly from the genital pore to the mid-field of the proglottid but it differs in that the ovary does not have straight lobes. The ovarian lobes of *O. saphena* are intermediate between the long wing-like bodies of *O. filaroides* and the long straight lobes of *O. cryptobranchi*. Although the ductus ejaculatorius in some instances coils within the base of the protruded cirrus, the coiling is not as extensive as that in *O. trimeresuri* and *O. marenzelleri*.

The work on this form was begun at the University of Michigan Biological Station, summer 1930, with the assistance of Dr. Lyell J. Thomas of the University of Illinois. It was completed during the fall semester at the University of Michigan under the direction of Dr. George R. La Rue. The author wishes to express her sincere appreciation and thanks to both for their generous assistance and kindly advice, without which the work could not have been done.

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## Explanation of Plate XIX

All drawings were made with the camera lucida. The projected scale in figures 1 and 7 represents 0.02 mm.; in all other figures, 0.2 mm.

Fig. 1.—Scolex of adult cestode; longitudinal section showing vestigial fifth sucker with basement membrane, nuclei, muscle fibers, tubular opening. Partial outlines of two other suckers are shown for reference.

Fig. 2.—Ventral aspect of protruded cirrus and vagina, showing ductus ejaculatorius coiled.

Fig. 3.—First proglottids.

Fig. 4.—Scolex of adult cestode from a toto mount.

Fig. 5.—Cross-section through gravid proglottid at level of cirrus-pouch, showing longitudinal muscles, vitellaria, lateral nerve, ventral excretory tube, testes, uterus, vas deferens, cirrus pouch and cirrus.

Fig. 6.—Cross-section through same proglottid as in Fig. 5, at level of ovary.

Fig. 7.—Oötype with Mehlis' gland (shell gland), oviduct, and beginning of uterine passage; frontal section.

Fig. 8.—Organs of interovarial space reconstructed from camera outlines. Dorsal side uppermost.

Fig. 9.—Mature proglottid, ventral aspect.

Fig. 10.—Ruptured proglottid, ventral aspect.

### Abbreviations Used

*c.* Cirrus
*e.* Ventral excretory tube
*i* Interovarian organs
*o.* Ovary
*u.* Uterus
*v.* Vitelline duct

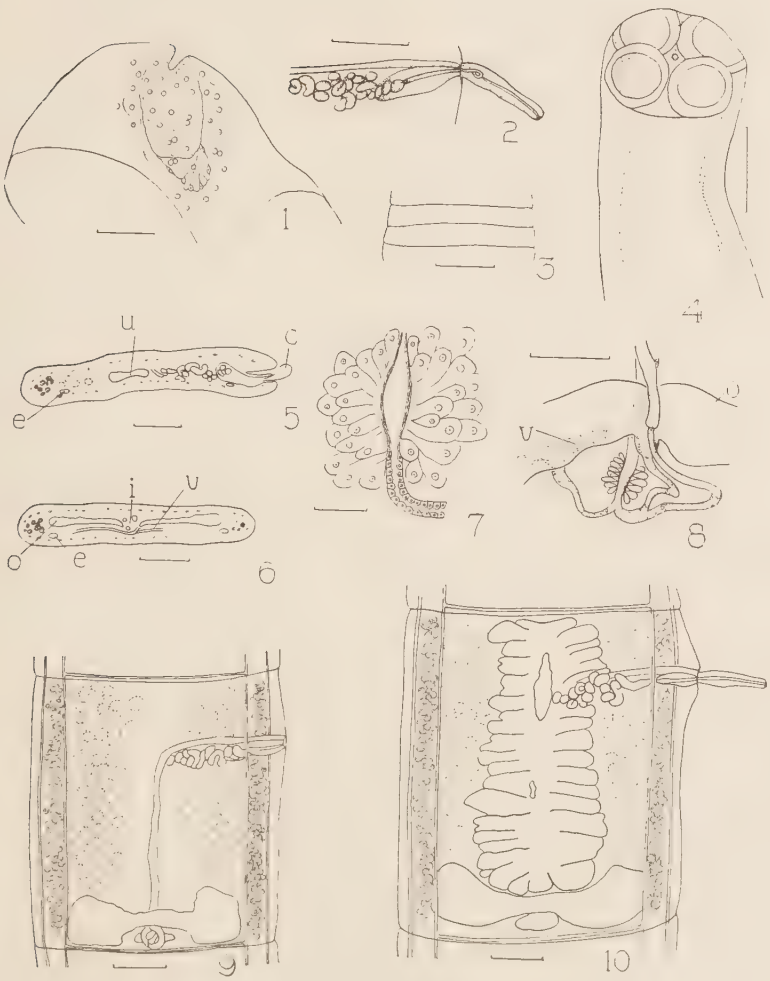


PLATE XIX

# NOTES ON THE LIFE HISTORY OF *OPHIOTAENIA SAPHENA* FROM *RANA CLAMITANS* LATR.

LYELL J. THOMAS

Fortner (1923) in his survey of the frog parasites of the Douglas Lake region, Cheboygan County, Michigan, during the summers of 1917 and 1919, mentions that of 208 frogs examined, ten per cent of the *Rana clamitans* taken in 1917 and one per cent of the *Rana pipiens* in 1919 contained tapes of the family Proteocephalidae. An examination of the literature on the tapeworms of amphibia, La Rue (1909, 1911, 1914 and 1914a), Johnston (1913), Jewell (1916), Dickey (1921), Woodland (1925), and Hannum (1925), directed the attention of the writer to the paucity of the work on the tapeworms of amphibia, especially of the Anura. This deficiency in the literature in view of the common use of frogs for some time throughout the world as objects for laboratory study and parasitological investigations can lead one to no other conclusion than that tapeworms of frogs are not common and that even less is known concerning their life histories.

During the routine examination of frogs by the class in helminthology at the University of Michigen Biological Station at Douglas Lake, Michigan, proteocephalids were found in *Rana clamitans* taken at Fontinalis Run, Burt Lake, July 3, 1930. Although more *Rana pipiens* than *R. clamitans* were examined, no tapes were found in the former. To a student Osler (1931), was given the task of working out the morphology of the adult worm an Ophiotaenia and the writer proceeded to unravel the life history. Eggs were shed in great numbers in the wash water and were set aside for infection experiments. The average measurements of the eggs are as follows: diameter of the outer gelatinous embryophore 46.7$\mu$; thickness of the outer gelatinous embryophore 10.8$\mu$; diameter of the unhatched onchosphere 17$\mu$. The hooks of the hexacanth embryo measure 6.3$\mu$ to 7.2$\mu$ in length. The majority of the eggs were not fully developed when shed in tap water (Fig. 1) but by July 6, three days later, all seemed to have onchospheres formed. In all eggs however taken from frog feces (Fig. 2) the embryos were complete.

## *Infection Experiments and Observations*

A medicine dropper containing one cc. of the wash water with eggs was added to each of twelve two inch stenders on July 9 and a mixed laboratory culture of copepods was introduced to each dish. On July 12 Paramecia were placed in the stender dishes to help clear up bacterial forms that were clouding the dishes and to furnish food

for the Cyclops. An examination of the copepods on July 16 showed eggs in the intestine but no onchospheres in the body cavity. *Mesocyclops obsoletus (C. leuckarti), Cyclops vulgaris (= C. viridis),* and *Macrocyclops annulicornis (= C. albidus)* were observed eating the eggs.

A *Rana clamitans* taken August 5 from Fontinalis Run harbored one immature tapeworm which measured 8.5 cm. in length. The stomach contents of the frog contained a partially digested beetle, *Harpulus* sp? and from the muscles of the thorax of this coleopteran thirty-nine cestode cysts were taken. The fact that some of these cysts were partially digested and all were inactive when dissected out, in addition to the length of the larval hooks which measured over ten microns, lead the writer to doubt their being stages of the same tape. Numerous acanthocephalan cysts were also noted in the mesenteries of this frog.

On August 11 field observations were made of the general region from which the frogs were obtained. Although *Rana pipiens* and *R. cantabrigensis* were also brought from the same locality and examined no tapeworms were present. Fontinalis Run as the name implies was once a good trout stream but has become blocked with débris so that the current is very sluggish where it flows through a straight man-made channel into Burt Lake. Nearby at a point where two highways intersect at right angles, the most frequent infections were present in frogs taken from the cold waters of narrow spring-fed side-ditches which empty into the lake. Of twenty *R. clamitans* from one ditch eight were infected and two of these harbored two adult tapeworms each. Syrphus-flies, beetles, *Harplus* sp?, *Dytiscus* sp?, *Gyrinus* sp?, occasional grasshoppers, a few Copepoda and Cladocera, as well as snails were present in the stomach contents. A juvenile tapeworm 3.937 mm. long (Fig. 3) was found in the intestinal wash from a *R. clamitans* examined August 14. The scolex was invaginated when first observed but after the specimen was placed on a slide in Ringer's solution and examined the scolex was evaginated and a fifth apical sucker was seen. This fifth sucker had a noticeable decrease in diameter when compared with each of the other four suckers and was very mobile. The cup of the sucker opened and closed so that the aperture was constantly changing. Again on August 15 two more immature tapeworms were taken from the intestine of a female *R. clamitans.* One of these had the scolex invaginated when first observed. The fully extended worm measured 1.925 mm. in length and was 0.525 mm. wide at the scolex end; the fifth sucker was approximately the same diameter as the others. The other specimen was 2.9 mm. in length and had a decided hypertrophy of the fifth sucker. A mature proteocephalid was quickly removed from a *R. clamitans* August 15 and placed in tap water where the eggs were shed in great numbers. One cc. of the eggs greatly diluted in tap water was added to each of twenty-four

two-inch stender dishes with ground glass covers. Gyrinid larvae and amphipods were added to twelve of the dishes and when examined August 19 were negative. A laboratory culture of Cyclops was added to the other twelve stender dishes and had to be examined August 19 due to the closing of the Biological Station. A *Cyclops vulgaris* was found with three hexacanth embryos in the body cavity. One measured 19.8$\mu$ in diameter and a clear bubble-like vesicle attached to it measured 26.1$\mu$ in diameter. The embryo was leach-like in its movements; the middle pair of hooks pulled in and extended rhythmically, the other two pairs synchronously but in the reverse order.

Eggs collected from two adult tapeworms removed from the small intestine of a *R. clamitans* August 17 were concentrated in ice cold tap water and placed in tightly corked two ounce vials. These were packed in cylindrical cardboard mailing cases and taken to Madison, Wisconsin, where the study was continued. On August 23, Cyclops were obtained from Lake Wingra, Wisconsin, and one hundred fifty of these copepods selected at random were inspected under the microscope for possible natural infection. They all appeared to be negative for tapes. Fifty were placed in a pint jar for controls. One hundred were divided into lots of twenty each in five pint jars with about four inches of filtered lake water in each jar. These containers were set in a cool light place in a basement room. Cyclops from jars Nos. 1 and 2 were examined microscopically August 25; five *Mesocyclops obsoletus* contained hexacanth embryos in the body cavity a little larger than unhatched embryos. One copepod of this lot had ten larval tapeworms in the body cavity which measured approximately 21.7$\mu$ by 10.8$\mu$; another had eleven in the body cavity with the following average measurements, 26$\mu$ by 10.8$\mu$; one six and one seven respectively in the body cavity; and another three in the abdominal cavity near the rectum. Five *Cyclops brevispinosus* were examined and the following observations were made: one had eight in the body cavity; two, nine each in the body cavity; one none; another eight in the thoracic region and six packed around the intestine. Measurements of three procercoids from these copepods were as follows: 26$\mu$ by 10.8$\mu$; 30.3$\mu$ by 10.8$\mu$; 21.7$\mu$ by 10.8$\mu$. One *Macrocyclops annulicornis* had twelve hexacanth embryos in the body cavity. Again on August 26 Cyclops were examined from jars Nos. 3, 4, and 5. Five *Cyclops brevispinosus* contained five, three, six, three, and seven respectively of the larval tapes. *Mesocyclops obsoletus* had heavy infections as follows: one fifteen, one five, one twelve, one twenty, and one twenty-two; one four; and one only two in the abdominal and anterior body region cavities respectively.

An observation was made beginning at 4:10 p.m. August 26 of an onchosphere passing through the intestine of a *M. obsoletus*. In this

copepod the intestine was constricted just anterior to the rectal region and the hexacanth embryo located within this constricted part was extremely active with its larval hooks. By 5:00 p.m. the embryo had practically made its way into the abdominal cavity and a clear bubble-like bladder (Fig. 4) seemed to be attached to the onchosphere by a delicate membrane. The elapsed time for the penetration was fifty minutes. A few Cyclops observed with tapeworms in the posterior body region were set apart in a separate jar and observed again on August 30. Most of the larval tapeworms had shifted by that time to the thoracic cavity. A *Cyclops brevispinosus* examined September 2 by dissection showed nine worms in the body cavity; one, larger than the others, measured 43.4$\mu$ in diameter. In addition to the larvae the body cavity was packed with flagellate protozoa. A *M. obsoletus* examined by dissection on this same date had ten larval tapeworms, three of which measured as follows: 21.7$\mu$; 30.3$\mu$; and 22.5$\mu$ respectively in diameter. It was also observed in this heavily infected Cyclops that the host tissues seemed to be disintegrating and during the normal movements of the digestive tract the procercoids shifted about from the anterior body region to the posterior body region like loose stones rolling about in the body cavity.

The sixth of September the first copepod removed from jar No. 3 was found to have two fully formed procercoids in the body cavity (Fig. 5) and one without a cercomer or scolex. The copepod was *Cyclops vulgaris* var. *brevispinosus*. Large calcareous bodies, fifteen to twenty in number were scattered about in the body proper of the procercoids and measured on the average 10.8$\mu$ in diameter. Some of these calcareous bodies were irregular in shape. In each of the mature procercoids (Fig. 7) the scolex was inverted and the cercomer with large transparent cells was very actively extended and contracted. The average measurements of these procercoids were as follows: length of body extended 162.7$\mu$; width of body extended 75.9$\mu$; cercomer extended 43.4$\mu$ in length and 30.3$\mu$ in width. The copepod containing these worms was very sluggish in its movements. The procercoids were dissected out in physiological salt solution and within a few minutes their scolices were everted (Fig. 6). The larval hooks were seen in the posterior part of the body proper and not in the cercomer. From another copepod of this same species another single procercoid was taken by dissection. Its cercomer was shed in salt solution (Fig. 8) when brought under pressure of a cover glass. In all other specimens examined the larval hooks were scattered about in the posterior part of the body and were not constricted off with the cercomer.

Thirty of the control copepods selected at random were dissected but no tapeworms were found when examined September 6, 1930.

DISCUSSION

In checking over the intermediate hosts examined it was found that *C. brevispinosus* was infected with an average of seven hexacanth embryos and *M. obsoletus* contained an average of twelve larval tapes. Although *M. obsoletus* seemed to eat more of the eggs the larvae did not seem to thrive so well in the body cavity as in the case of *C. brevispinosus*. This may have been due partly to too heavy infections. The scolex and main trunk of the procercoids resembled in all details the youngest forms removed from the intestine of *Rana clamitans*. Those in the final host however had increased greatly in nearly all proportions. These observations directed the writer to consider further the data at hand.

Fortner (1923) records that in the Douglas Lake region of one hundred seventy-seven specimens of *Rana pipiens*, twenty-nine *Rana clamitans*, and two *Rana cantabrigensis* examined, none of the *R. cantabrigensis* had proteocephalids, while ten per cent of the *R. clamitans* in 1917 were infected and one per cent of the *R. pipiens* in 1919 harbored tapeworms. These collections of infected frogs were taken from Maple River which flows into Douglas Lake; South Fish Tail Bay with scant water plants and shallows; and Sunny Strand marked by a sedge covered beach and shallow water, both shore line areas of Douglas Lake. Although classes in helminthology have collected from these general regions since 1927 no tapeworms of frogs have come to the attention of the writer. Seven per cent of the frogs from Maple River and eleven per cent of the frogs from South Fish Tail Bay had tapeworms in 1917. None were found in frogs from Maple River and South Fish Tail Bay in 1919 but a twenty five per cent infestation was present in frogs taken at Sunny Strand. Comparing this data with that obtained by the writer and students in the summer of 1930 it is interesting to note that twenty-eight tapeworms were obtained from 97 *R. clamitans* in a rather restricted area at Fontinalis Run or a 28.8 per cent infection. None were found in *R. pipiens* or *R. cantabrigensis* from the same region. The infected frogs ranged in size from three inches in length to seven inches in length, from newly metamorphosed individuals to fully developed adults. *R. clamitans* was always found in the vegetation along the margins of the ditches or in mid stream among vegetation masses. The other species of frogs were found in numbers in the grass along the ditches and in the boggy but grassy clearings back several yards from the roads and ditches. These observations are in accord with those of Fortner (1923). Cyclops were found most abundantly in shallow water in the masses of vegetation along the stream margin or in dense vegetation out in the stream. Immature tapeworms 1.925 mm. long to 3.937 mm. in length were found in sexually mature

frogs, and adults were present in newly metamorphosed frogs. An examination of *R. clamitans* tadpoles showed Copepoda and Cladocera mixed in with the vegetable débris of the intestine. The fifth sucker in the mature procercoids is the same diameter as that of each of the other four suckers. In the youngest tapeworms taken from *R. clamitans* the sucker diameters were alike in all five suckers. In slightly larger plerocercoids (Fig. 11) the fifth sucker is greater in diameter and with heavier musculature than in the other four suckers. This apical fifth sucker in both the procercoids and juvenile tapeworms is well supplied with sphincter muscles and can close the aperture to a minute opening as is shown (Figs. 3 and 11) by an observation of the living animal. La Rue (1909: 41) notes that in the plerocercoids of *O. filaroides* the fifth sucker, or as he calls it "end-organ, is much reduced in size" and "the end-organ in the adult scolex is small and may not be seen in toto mounts." His Plate I, fig. 4 shows the hypertrophy of the fifth sucker, also the invagination of the scolex. Woodland (1925c: 371) says regarding an apical structure on the scolex of *Proteocephalus tigrinus* from the common Indian frog, *Rana tigrina,* "The apex of the scolex is slightly prominent and bears a spherical organ, with very thick walls, small slit-like lumen and an opening at the extremity. I cannot speak as to its nature but it is apparently neither a functional apical sucker nor a mere 'muscle plug' remnant of a muscular rostellum." From this description and from an examination of his drawings, the writer believes this is a vestigial fifth sucker such as is present in *O. filaroides* La Rue and in *Ophiotaenia saphena* Osler (1931). Hungerbühler (1910) does not figure the scolex of (*Ichthyotaenia schultzei*) a proteocephalid from the South African frog, *Rana adspersa,* but notes that no fifth apical sucker is present. In one immature tapeworm, 3.937 mm. in length (Fig. 3) taken August 14 from a *R. clamitans* the apical sucker was already beginning to lag behind in development. By the time the adult stage is reached (Fig. 9) this sucker is exceedingly small, 0.049 mm. by 0.023 mm., in comparison with the other four suckers, each of whose diameters measure about 0.135 mm.

In only a few proteocephalids thus far described is the scolex of the procercoid invaginated when in the body cavity of the Cyclops. Bangham (1925) and Hunter (1928) have figured and described this condition in *Proteocephalus ambloplitis.* Essex (1927) also shows this to be the state of the procercoids of *Corallobothrium fimbriatum* and *C. giganteum.* Kuczkowski (1925) has shown that the larval hooks of *Proteocephalus (Ichthyotaenia) percae* Müll. do not constrict off with the cercomer but remain in the body proper. Essex (1927) says that in Corallobothrium the hooks may or may not be constricted off with the cercomer. This same author has described a pseudophyllidean, *Diphyllobothrium latum* with the hooks partly in the body of the procercoid

and partly in the cercomer. Hunter (1928) shows the larval hooklets distributed about in the body of the procercoid of *P. ambloplitis* but no cercomer is present. In the procercoid of *P. pinguis* La Rue, Hunter (1929) has described a fifth sucker without the invagination of the scolex and no cercomer is present. Bangham (1925) figures a fifth sucker for *P. pearsei* and no cercomer. It is at present difficult to say just how much value these different developmental features are to the taxonomist. Perhaps the Proteocephalidae have a rather mixed ancestry or it may be that their genes are in a rather unstable evolutionary state.

The rather low infection rate of the parasite in *Rana clamitans*, 28.8 per cent, may possibly be accounted for by the occasional and accidental ingestion of copepods which are sluggish in their reactions when parasitized. The writer has seen these frogs partly submerged along the margin of the stream take Gyrinid beetles that came within range. Parasitized copepods might easily be taken in accidentally with such forms that frequent the water. The lower infection rate in *Rana pipiens* may be possibly accounted for by their tendency to wander farther away from the water in the search for food.

Direct infection by means of a parasitized copepod without a second intermediate host is not unusual among proteocephalids. Since the first demonstration of a tapeworm life history through the agency of a copepod by Meggitt (1914) this seems to be the usual method for this group. The presence of microcrustacea in the digestive tracts of both tadpoles and adult frogs, the similarity of procercoids and juvenile tapeworms, the habits of *Rana clamitans* and of the copepods, the higher infection rate in this species of frog correlated with the habitat, are facts exceedingly suggestive that the frogs become infected directly by accidentally ingesting parasitized Cyclops. This hypothesis however may only be substantiated by carefully controlled feeding experiments which the writer plans to continue another summer.

## SUMMARY

1. The eggs of a new species, *Ophiotaenia saphena* Osler, from *Rana clamitans* when shed in tap water have immature onchospheres in many of them but development is completed within three days.

2. Eggs taken from frog feces had completely formed onchospheres.

3. *Mesocyclops obsoletus (= Cyclops leuckarti)*, *Macrocyclops annulicornis (=Cyclops albidus)*, or *Cyclops vulgaris (= C. viridis)* may ingest the eggs but *Cyclops vulgaris* var. *brevispinosus* was the only copepod found with mature procercoids.

4. Twenty-two immature procercoids were found in one *M. obsoletus*.

5. About fifty minutes were required for an onchosphere to penetrate the intestine of a Cyclops.

6. Onchospheres in the posterior body cavity may shift about and come to lie in the anterior body cavity.

7. Twelve to fourteen days after the ingestion of eggs, mature procercoids are found in the body cavity of Cyclops.

8. The larval hooklets remain in the body proper of the procercoid and are not constricted off with the cercomer.

9. The cercomer is shed in physiological salt solution.

10. A fully formed scolex with a functional fifth sucker is invaginated when the procercoid is within the copepod.

11. The youngest plerocercoids from the small intestine of *Rana clamitans* are similar to the procercoids and invert the scolex.

12. The functional fifth sucker in the procercoid hypertrophies in the plerocercoid, then atrophies, and finally becomes vestigial in the adult worm.

13. Copepoda, Cladocera, and aquatic beetles as well as snails are found in the intestinal débris of the frogs.

14. The habitats of *Rana clamitans* and the Copepoda are similar and dovetail.

15. Infection rates in frogs correspond with their habits.

16. Direct infection of frogs by accidental eating of infected Cyclops is suggested.

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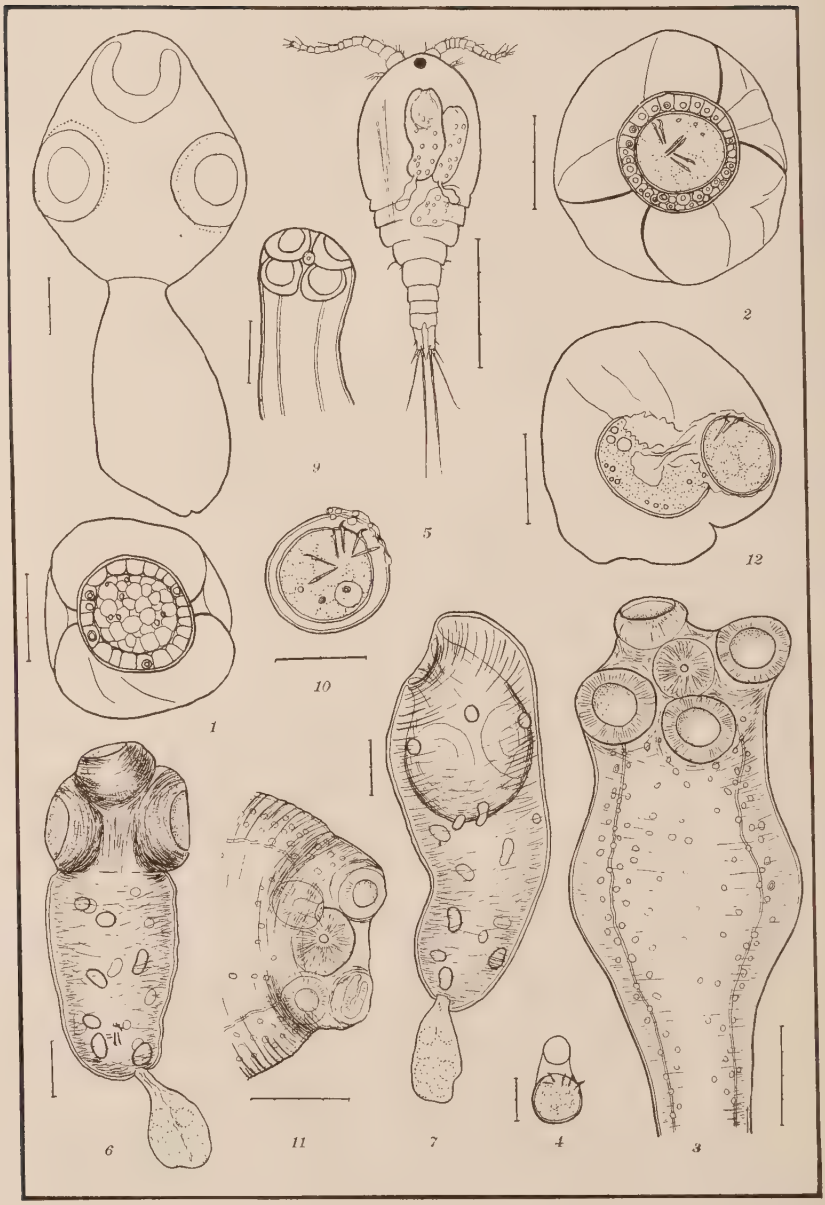


PLATE XX

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## Explanation of Plate XX

All drawings except figure 9 were made from living material and with the aid of the camera lucida. The scale in figures 3, 5 and 11 represents 0.20 mm.; in all other figures it is 0.02 mm.

Fig. 1.—Egg of *Ophiotaenia saphena* Osler from *Rana clamitans* shed in tap water showing undeveloped onchosphere.

Fig. 2.—A fully developed egg with outer gelatinous embryophore and membranes.

Fig. 3.—Juvenile tapeworm from the small intestine of *Rana clamitans* showing beginning atrophy of the fifth sucker and aperture nearly closed, also heavy muscles of the suckers and excretory ducts.

Fig. 4.—Onchosphere, shortly after penetration into the body cavity of Cyclops, with clear bubble-like attachment.

Fig. 5.—*Cyclops vulgaris* var. *brevispinosus* with one undeveloped and two mature procercoids in the body cavity.

Fig. 6.—Mature procercoid with scolex evaginated in physiological salt solution showing apical fifth sucker, hooks in the posterior part of the body and none in the cercomer.

Fig. 7.—Mature procercoid from body cavity of Cyclops with scolex invaginated; large calcareous bodies present.

Fig. 8.—Procercoid under pressure of the coverglass with cercomer shed in physiological salt solution. All suckers equal.

Fig. 9.—Totomount, scolex of the adult tapeworm showing vestigial fifth sucker. From Osler.

Fig. 10.—Onchosphere within the inner embryonic membrane, from the intestine of Cyclops.

Fig. 11.—Plerocercoid scolex showing hypertrophy of fifth sucker.

Fig. 12.—A freshly ingested egg removed from the intestine of *Mesocyclops obsoletus.*

# STUDIES ON THE ACTIVITY OF THE INFECTIVE LARVAE OF THE RAT STRONGYLID, *NIPPOSTRONGYLUS MURIS* *

CANDIDO M. AFRICA
University of the Philippines

Yokogawa (1920) described the nematode which is now known as *Nippostrongylus muris* from the intestine of wild rats. Two years later the same author published an admirable paper on the development of this worm from the egg to the adult stage (Yokogawa, 1922). He found that under favorable conditions the eggs hatch in about 20 to 24 hours after being passed with the feces. The hatched larvae undergo only one molt outside the host, after which the infective filariform stages are usually found at the edge of the culture medium or on the wall of the container five days after the cultures are made. Change of habitat brought about by entrance into the host separates the second from the third stages which occur inside the host. Sexual maturity is attained in from five to six days after skin penetration as evidenced by the appearance of the eggs in the feces. Rats can be infected either by way of the mouth or through the skin although the latter is by far the more effective method. The same author also made studies on the influence of air, light, moisture and temperature on the activity of the free living stages, but his observations along these lines are far from being complete. Therefore, further studies on the biology of the free living stages of this nematode are desirable.

In the researches which will be discussed in this paper experiments were made to test the activities of the infective stages of *N. muris* in the soil, such points being considered as lateral and vertical migrations, crossing of water barriers, and behavior under the influence of different stimuli. Observations were also made on the manner by which the larvae effect their progress during their migration as well as on the manner by which they maintain their infective positions in the soil. Carefully controlled experiments were also made on the effect of low temperatures on the hatching of the eggs and the development of the larvae. The effect of prolonged exposures to low temperatures on the vitality of the infective stages was also tested.

The strain used in this work was supplied the writer by Mr. G. Winfield of the Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University, who obtained it from

* From the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University.

wild rats around Baltimore, Maryland. Yokogawa's (1922) filter paper method of culture was used to keep the strain going, while soil cultures were resorted to in the study of the larval behavior. Various other culture methods were also tried. The descriptions of the materials and methods used in the various experiments in this study are so intimately connected with the discussion of each experiment that it is deemed convenient to describe them later.

## EXPERIMENTS ON LATERAL MIGRATION

It was noted by Yokogawa (1922) and confirmed in the present study that in solid cultures the larvae after the first molt tend to leave the fecal smear and migrate towards the edge of the moist filter paper, or even climb up the sides of the container as far as the moisture present permits. This migratory proclivity in solid media was also previously observed by Theiler and Robertson (1915) and by Veglia (1915) for the wire-worm of the ostrich and for *Hacmonchus contortus* respectively. In order to test whether the larvae of *N. muris* can effect a similar migration in the soil, the following experiment was performed:

A metal pan about 9½ inches in diameter and about an inch in height was half-filled with garden soil which was previously made "sterile" for free living nematodes. The soil was then sprayed evenly with enough water to moisten it just a little below saturation. A circular piece of filter paper about an inch in diameter previously moistened and smeared with freshly passed feces richly laden with eggs, was placed in the center of the soil as the zone of infestation. The pan was then placed in a moist chamber, which was set aside at room temperature. A control pan prepared in the same manner and infested with eggs from the same source, but left uncovered was also made. On examining the first pan on the third day by means of a hand lens, larvae were found crawling all over the surface of the soil surrounding the zone of infestation. No congregation or clumping of larvae was found at this time. On the fifth day clumps or "colonies" of larvae were noted especially on prominences such as lumps of soil and pieces of stick that extended out from the general surface. On the sixth day "colonies" appeared even on the sides of the container as whitish patches, extending up as far as from one to two centimeters. It was noted that very few larvae were found in depressions or furrows on the soil. They seemed to collect in places which were more exposed to the air. If the soil was upturned, they reappeared very quickly at the surface. After the seventh day, the soil especially at the edge near the sides of the container, became so highly infested that the soil clumps and pieces of stick that extended out from the surface became literally covered with whitish velvety projections that waved in the air.

On examining one of these projections it was found to consist of many individual larvae that had intimately coiled themselves up together, forming a sort of miniature whorl with their tails anchored at the surface of the soil. Larvae were also seen climbing up to the top of tiny plants that had sprouted from the soil. On examining the control pan in the same manner, practically no migration at all was noted. The hatched larvae in this case were found almost entirely confined to the zone of infestation, although a few were noted in the soil of the immediate vicinity.

The above experiment demonstrates that the larvae of this worm, unlike those of the hookworm (Augustine, 1923), can effect a lateral migration under certain conditions, of which perhaps the most important or essential is the degree of moisture present. How far they can actually migrate from the focus of infestation has not been determined in this work, but it has been shown that they could negotiate with ease at least a distance of four inches from the zone of infestation to the sides of the container. That they can travel in moist soil provided the moisture is uniform and the temperature favorable, many times this distance, seems very likely. The fact that in the uncovered pan no appreciable migration was noted in spite of the fact that the soil was sprayed daily, shows that the moisture must be uniform in the ambient before they can effect migration. This is significant considering the fact that inside rat holes and burrows, a condition of humidity such as that obtained in the moist chamber is probably usually found.

### VERTICAL MIGRATION

The next question that naturally came to mind was whether the larvae perform vertical migration. Payne (1922, 1923 a and b) conclusively demonstrated by laboratory and field experiments that hookworm larvae can effect a vertical upward migration, a distance of from 3 to 4 inches under laboratory conditions and at least up to 36 inches in sandy loam under outdoor conditions. To test whether the larvae of *N. muris* can effect a similar migration a glass cylinder about 12 inches long and 2 inches in diameter and open at both ends, was filled with garden soil which had been previously "sterilized." The contents were then moistened just below saturation, and at one end pieces of moist filter paper previously smeared with freshly passed feces rich in eggs were buried to a distance of four inches and then this end was plugged with filter paper to prevent the soil from being washed away. The cylinder was then set up vertically in the center of a glass jar containing about one-half inch of water, which was daily replenished. The bottom of a small beaker was stuffed with filter paper saturated with moisture, and this was used to cover the open

end of the cylinder in order to keep the moisture in the soil as uniform as possible. The preparation was set aside at room temperature. At the end of 12 days "colonies" of larvae were found on the surface of the soil. An attempt to duplicate this experiment was rewarded with success, although at this time no "colonies" were found on the top of the cultures, only a few larvae being recovered by the use of the Baermann apparatus. This indicates that an optimum degree of moisture is required by the larvae to effect a vertical migration, just as in the lateral one. Payne (1923 b) pointed this out previously for hookworm larvae. The relationship between this moisture condition and the migratory capacity of the larvae is perhaps so delicate that a very slight variation from the optimum is enough to retard or arrest their progress.

### MIGRATION THROUGH WATER BARRIER

Intrigued by the manifest activity of the larvae of this form, the question as to whether they can cross water barriers occurred to the writer's mind. In order to test this point the following experiment was performed:

A large Petri dish about 5½ inches in diameter and one half-inch in height was half-filled with garden soil previously "sterilized" and on top of this at the center was placed a circular piece of moist filter paper about one-half inch in diameter, previously smeared with fresh feces richly laden with eggs. This preparation was then placed in the center of a large glass jar containing enough water so that when the Petri dish was placed in position in the center, a water trap about two centimeters deep and about two inches wide was formed around it. The preparation was then covered and set aside at room temperature. On the sixth day when the culture was examined, "colonies" of larvae were found not only on the sides of the Petri dish but also on the inner wall of the glass jar to a distance of a few centimeters upwards. It was evident that the larvae had not only scaled the wall of the Petri dish, but had also managed to crawl down on its outer side into the water, crossed it and then climbed up the sides of the outer jar. As these larvae sink to the substratum, they must have crossed the "trap" not by swimming but by crawling movements at the bottom. It seems probable that they can do the same thing on rock or soil substratum instead of glass.

### LONGEVITY OF THE SECOND STAGE LARVAE IN WATER

On withdrawing a portion of the sediment at the bottom of the trap in the experiment just described it was found to be teeming with larvae in the second stage, the great majority of which still

retained their old cuticula. Apparently they were either those on their way to the outer wall, or those less enterprising ones which were not able to negotiate the distance. A portion of this sediment containing numerous larvae was introduced into a container with about two inches of water. This was set aside at room temperature uncovered but replenished with water every day to maintain its depth. At the end of ten days practically all the larvae were still alive and active. The larvae were then centrifuged and concentrated in a few drops of water, and then placed in a circular piece of filter paper which was in turn placed in the center of a soil culture in a moist chamber. After 48 hours the larvae were found to have migrated to the edge of the culture medium. This finding suggests that larvae that are washed into water collections may live there for a considerable period, and that as soon as these water holes dry up, they can again migrate to vantage points for an opportunity to infest hosts that may be passing by.

### BEHAVIOR OF THE INFECTIVE LARVAE IN THE SOIL

If an individual larva is watched by means of a hand lens during its migration, it will be seen that progress is effected by crawling movements from one soil particle to another much in the same manner as described by Payne (1923) for the hookworm larva in its vertical upward migration. In its progress the larva is usually seen extending the anterior part of its body, and searching now here, now there, for another soil particle or other object. In this position the larva is evidently kept in place by the film of moisture which surrounds the soil particle, a force so strong that the larva may be seen to extend more than two-thirds its body length without being dislodged. As contact between the moisture film of one soil particle that holds the larva and that of a neighboring one is not so readily established, it is amazing how much energy the larva can spare in this constant searching motion. As soon as contact is established and the moisture films of the two particles become continuous the larva can move from one to the other. Although there is not enough data to warrant a safe conclusion, it can be predicted with a reasonable degree of certainty that as in hookworm larvae they are enabled to move freely by the presence of an optimum amount of moisture, are arrested in their progress by a reduction of moisture, and apparently not able to make much headway in the presence of excessive moisture.

It has been previously mentioned that if the soil culture is examined sometime before the fifth day after the culture is made, the larvae can be seen more or less evenly distributed over the entire field, whereas if examined on the fifth day, or thereafter, the migrating larvae congregate and form "colonies" on prominences and objects that extend

from the surface. What attracts them to such points is not definitely known. Lane (1930) in the study of the behavior of hookworm larvae in deep glass cells found that these creatures tend to congregate after some time at the angles of the cell formed by the sides. He attributed this to purely mechanical factors and not to any form of taxis. Whether the larvae of *N. muris* are attracted to prominences and objects that extend from the surface by thigmotaxis or other forms of taxis, or whether they congregate there as a result of some purely mechanical force, is a matter of conjecture, but it seems more likely by reasoning from natural grounds, that they congregate at these points, because in that way they can with the best of advantage infect a host that may be passing by, than were they situated in depressions or crevices.

If a portion of the infested soil is transferred carefully from the culture to a slide and then examined under the low power binocular after it has been allowed to stand undisturbed for a few minutes in somber diffused light, the behavior of the larva as it probably occurs in nature may be studied with advantage. Slight mechanical disturbances such as jarring the table, tapping the slide gently, etc., are sufficient to stimulate the larvae to activity. In cultures more than six or seven days old, the cuticula in the majority of cases is slit open in a V-shaped fashion at the anterior end, which is invariably the end pointed out into the air. The posterior end, which is bent like a hook, is used as an anchor that fastens the larva to the soil. When free from disturbance the larva usually remains quiet inside the sheath, but the slightest stimulus from the ambient awakens it to activity. It then extends its anterior end into the air, the degree of extension being apparently directly proportional to the amount of stimulus applied. Sometimes more than half the body of the larva is extended out in a searching motion, but on withdrawing the source of stimulus, such as the heated point of a needle, the larva retreats back inside the cuticula which appears to be a rigid structure housing the larva. If enough heat is applied, the larvae are stimulated to violent activity, some of them being literally catapulted from their sheaths. Those that extend out their bodies for a considerable distance become entangled with their neighbors so that oftentimes a mass of wriggling creatures hangs dangling in the air, suspended from the empty "shells" which they have deserted. Provided the culture is not disturbed, the larvae remain inside their sheaths for a considerable length of time, and although they are apparently free to leave them at any time they remain inside unless stimulated. In a soil culture 35 days old, which has been left undisturbed in a moist chamber, the majority of the larvae were found still maintaining their original position in the soil, and still possessed of their sheaths, which by this time, however, had

contracted considerably, thus exposing the greater part of the bodies of the larvae. The sheaths are whitish in color originally, but they turn brown with age, so that old colonies appear like patches of rust. Then, in very old cultures, the sheaths contract until only the tail end of the larvae is covered. This explains why the "velvety" covering of the infested soil appears white when the culture is young, turns brown as it grows older and then turns white again when the sheaths have contracted, exposing the greater portion of the body of the larvae which refuse to leave them.

### REMIGRATION OF THE LARVAE

To test whether the second stage larvae that had succeeded in reaching the edge of the culture and had assumed their infective position in the soil can remigrate if placed in disadvantageous situations, larvae were removed from the infested soil particles at the edge of a six day old culture as well as from the sides of the dish. Then after concentrating them in a few drops of water, they were transferred onto a circular piece of filter paper about the size of a half dollar which had been previously placed in the center of a "sterile" soil, moistened just as in the previous migration experiments. When the culture was examined 48 hours after, a large number of these larvae had migrated to the edge of the culture medium, assuming positions similar to those in the previous culture. These larvae were found to retain their sheaths, whereas those that were still found in the zone of infestation or on its immediate environs were all naked. Since in the process of transfer while in a liquid medium, a proportion of the larvae succeeded in escaping from their sheaths, it was evident that these freed larvae were the ones left behind in the zone of infestation, being unable to effect a remigration. Apparently they had lost their migratory instinct or had become too weak for further activity.

### REACTION TO DIFFUSED SUNLIGHT

To test the reaction of the larvae to diffused sunlight, a soil culture was made in the same manner as before in a large Petri dish, half of which was shaded with black paper and placed in a moist chamber. The preparation was placed near a window in such a way that while it received plenty of diffused sunlight no direct sun rays fell on it. An unshaded control culture prepared in the same manner was placed beside it. After six days both cultures were examined under the binocular, and it was found that in the shaded culture most of the larval "colonies" were found under the shaded area on soil clumps near the wall of the container. The soil contents of the Petri dish

were bisected, and each portion placed in Baermann's apparatus to recover the larvae. Sixty-seven thousand larvae were recovered from the shaded portion, whereas only 6,000 larvae were recovered from the unshaded area. In the control culture the uniform distribution of the "colonies" around the edge of the soil was so apparent that no counts were deemed necessary. This demonstrates that the larvae of *N. muris* are negatively heliotropic and have a preference for dark places. This is significant since the natural haunts of wild rats are usually inaccessible to sunlight.

### EFFECT OF COLD ON EGGS AND LARVAE

To test the effect of low temperatures on the hatching of the eggs and development of the hatched larvae two preliminary experiments were made. Two Petri dish cultures were made after Yokogawa's method from feces collected from the same rat. Culture A was placed in the ice-box * and culture B was set aside at room temperature on February 2. After 24 hours the cultures were examined. The majority of the eggs in the culture allowed to develop at room temperature had hatched, while no hatched larvae were found in culture A after a prolonged search. Practically no development took place in the ice-box culture as most of the eggs were still either in the blastomere or morula stage, very few reaching even the "tadpole" stage. On February 3 culture A was allowed to develop at room temperature and after 24 hours it was again examined. The majority of the eggs were found to have hatched this time, the larvae were all alive and active, and no difference in the degree of development was noted between them and those hatched in the control culture. The control culture was also examined and the larvae were found to be undergoing the first molt. Culture A was again placed in the ice-box for the next twenty-four hours and again examined after the lapse of that time. The larvae that hatched previously were found dead. After examining this culture it was again placed at room temperature, and again examined the following day. Many hatched larvae were again noted. It was then allowed to continue development at room temperature as the culture B. On February 9 both cultures were examined. At this time the second stage larvae had left the fecal smear and migrated to the edge of the filter paper. In culture A only a few larvae were seen to have collected at the edge of the filter paper and practically none on the sides of the dish save for a few strayed individual ones. In culture B clumps of larvae were found collecting in numbers both on the edges of the filter paper and on the sides of the container. On the twelfth

* The temperature of the ice-box used in this experiment is 33°-34°F. above zero during the day and around 45°F. from midnight to the next morning.

day both cultures were placed in the ice-box and left there for twenty-four hours. At the end of that time both were examined and the larvae were found practically all alive and active. Infestation experiments on two young rats proved that the larvae from both cultures were infective.

The results of this experiment indicate that exposure to temperatures ranging from 33° to 45° F. for twenty-four hours completely arrests the development of the eggs of this worm, kills the newly hatched larvae, but apparently has no serious effect on the infective stages or second stage larvae.

After having determined that short exposures to ice-box temperature apparently had no effect on the infective larvae, an experiment was made to find out the effect of more prolonged exposures. To

TABLE 1.—*Showing the Effect of Exposure to Ice-Box Temperatures (33° to 45°F.) on the Vitality of the Infective Stage of* N. muris

| | Larvae Dead | | Larvae Living | | Total Examined | |
|---|---|---|---|---|---|---|
| Date of Examination | Ice-box Culture | Room Temperature Culture | Ice-box Culture | Room Temperature Culture | Ice-box Culture | Room Temperature Culture |
| February 23 | 7 | 0 | 383 | 480 | 390 | 480 |
| February 24 | 13 | 0 | 430 | 380 | 443 | 380 |
| February 25 | 16 | 0 | 520 | 546 | 536 | 546 |
| February 26 | 38 | 1 | 360 | 406 | 398 | 407 |
| February 27 | 51 | 0 | 395 | 390 | 446 | 390 |
| February 28 | 85 | 6 | 380 | 445 | 465 | 451 |
| March 2 | 304 | 7 | 204 | 467 | 508 | 474 |
| March 3 | 375 | 16 | 125 | 423 | 500 | 439 |
| March 4 | 306 | 13 | 214 | 365 | 520 | 378 |
| March 5 | 406 | 27 | 123 | 416 | 529 | 443 |

attain this end, ten small glass dishes each provided with a cover and a floor of moist filter paper were prepared. In each was placed a lump of infested soil covered with larval "colonies." They were then placed in the ice-box on February 20. Similar preparations were placed at room temperature for controls. Starting from February 23, the dishes both in the ice-box and those placed at room temperature were examined one after another at one day intervals until the ten dishes in each set were finished. In this process a certain number of larvae were removed from each dish, and then the dead and living larvae were counted. The results of these observations are shown in Table 1.

As shown in Table 1 the mortality of the infective larvae was very much greater among those exposed to ice-box temperature than among those placed at room temperature. To test the infectivity of the larvae, two infestation experiments were performed. One young rat was infected through the skin with 200 living larvae obtained from the

dish exposed in the ice-box for five days. Another was infected in a similar manner with 200 living larvae from the dish exposed to room temperature for the same period. Autopsy at the height of infestation gave the following results: Rat infested with larvae exposed to ice-box temperature for five days gave 21 males and 37 females; control gave 30 males and 39 females. The same experiment was repeated for larvae exposed for 13 days with the following results: Rat infested with larvae from ice-box, no adults recovered; rat infested with larvae exposed to room temperature, 38 males and 58 females.

The results of these experiments show that while short exposures to cold temperature such as that obtained in the ice-box apparently have little or no effect on the vitality of the infective larvae, prolonged and continuous exposures to the same not only kill them but also nullify the infectivity of the survivors.

## SUMMARY

Studies on the biology of the free living stages of *Nippostrongylus muris* have been made with the following results:

1. In soil kept in moist chamber, the second stage larvae can effect a lateral migration, the distance actually covered being about four inches. That they can travel distances many times greater is highly probable. Larvae that have assumed their infective positions in the soil can remigrate if placed in an unfavorable situation.

2. The larvae can effect a vertical upward migration under laboratory conditions for at least eight inches. Apparently an optimum amount of moisture is essential for this migration just as for the lateral one. The relationship between this moisture condition and the migratory capacity of the larvae is perhaps so delicate that a very slight deviation from the optimum is enough to arrest their progress.

3. The infective stages can cross a water barrier of at least two inches on a glass substratum.

4. The infective stages can live under water two inches deep for at least ten days without losing their migratory capacity.

5. The larvae are negatively heliothropic and have preference for dark places. This is significant since the natural haunts of wild rats are usually inaccessible to sunlight.

6. The infective larvae, although essentially not enclosed in a sheath, is, strictly speaking, protected by the old cuticula which it refuses to leave unless stimulated.

7. Twenty-four hour exposures of the cultures to ice-box temperature, 33° to 45° F. completely arrests the development of the eggs, kills the newly hatched larvae, but apparently has no serious effect on the

infective stages. More prolonged and continuous exposure to the same temperature not only kills most of the larvae but also nullifies the infectivity of the survivors.

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# A COMPARATIVE STUDY OF MEASUREMENTS OF STAINED AND UNSTAINED CYSTS OF *GIARDIA LAMBLIA*

H. TSUCHIYA
The School of Hygiene and Public Health, Johns Hopkins University

Dobell and Jepps (1918) made a careful study of possible variations in the size of *E. histolytica* in direct and stained specimens, and found that there was at least a difference of ten per cent between the two sets of measurements. They advanced the idea that an addition of 10 per cent seemed justified when stained cysts were considered. Since the cyst of *Giardia lamblia* is quite similar in size to that of *E. histolytica,* it is conceivable that such a relation may occur here also.

*Measurements of Stained and Unstained Cysts of* Giardia lamblia *(100 Cysts Measured for Each Specimen)*

| | Length (Microns) | | Breadth (Microns) | | Volume (Cubic Microns) | | Ratio Between Unstained and Stained | | |
|---|---|---|---|---|---|---|---|---|---|
| Sample | Unstained | Stained | Unstained | Stained | Unstained | Stained | Length | Breadth | Volume |
| 1 | 14.73 | 13.22 | 10.04 | 8.79 | 761 | 525 | 1.11:1 | 1.14:1 | 1.45:1 |
| 2 | 14.73 | 13.83 | 10.30 | 9.17 | 801 | 598 | 1.07:1 | 1.12:1 | 1.34:1 |
| 3 | 14.17 | 12.71 | 10.01 | 9.30 | 730 | 564 | 1.11:1 | 1.08:1 | 1.29:1 |
| 4 | 13.73 | 13.07 | 9.96 | 9.20 | 698 | 567 | 1.05:1 | 1.09:1 | 1.23:1 |
| 5 | 13.92 | 12.79 | 9.55 | 9.02 | 647 | 534 | 1.09:1 | 1.06:1 | 1.21:1 |
| Total | 71.28 | 65.62 | 49.86 | 45.48 | 3,637 | 2,788 | 5.43:1 | 5.49:1 | 6.52:1 |
| Average | 14.26 | 12.72 | 9.97 | 9.10 | 727 | 558 | 1.09:1 | 1.10:1 | 1.30:1 |

In order to substantiate the above contention, the following study was carried out to determine the relative proportion in size between cysts stained by the iron hematoxylin method and those observed in direct smear examination. Five samples of stool were chosen at random, and the measurements of one hundred cysts each were taken, according to the method previously described. Briefly stated, the measurement was made from a camera lucida drawing using a 10 × eyepiece and 1.9 mm. oil immersion objective. The maximum length and breath of each cyst expressed in millimeters were later converted into microns. For the determination of the mean of length and breadth, the formula, $M = \frac{\text{ext}}{n}$ was used in which $n$ indicates the number of samples, $x$ the measurements of all samples and $f$ the frequencies in which they appear. The volume of the cyst was calculated by the formula $\frac{4}{3}\pi ab^2$ in which $a$ is ½ of the mean length and $b$ ½ of the mean breadth. the value of $\pi$ is known to be 3.1416.

According to the findings shown above it is evident that the difference in ratio between stained and unstained specimens lies within the range of from 0.05 to 0.11 in case of length, 0.06 to 0.14 in breadth and 0.21 to 0.45 in volume. The average difference expressed in terms of percentage would be, therefore, 9% in length, 10% in breadth and 30% in volume. This result seemed to prove conclusively that Dobell's contention could be taken as a means of determining the true measurements of the cysts of *Giardia lamblia*. Attention, however, may be called to the importance of such application especially in case cysts are so few that a concentration method of some sort is necessary in order to obtain a sufficient number for measurements.

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# THE RATE OF LOSS OF HOOKWORMS IN THE ABSENCE OF REINFESTATION*

FRED C. CALDWELL
AND
ELFREDA L. CALDWELL

The fundamental policies that have been followed in the control of hookworm disease have been based upon certain biological characteristics of the parasite. Epidemiological and laboratory evidence has seemed to indicate that hookworms, once acquired, are slowly lost, their life span extending over a period of years. It appeared to be necessary, therefore, in order to lessen the heavy burden produced by the parasite, to develop a general campaign of treatment of the infested population in conjunction with the slower, more permanent methods of control—sanitation and education.

Chandler, in his discussion of the rates of acquisition and loss of hookworms (1929), questions the validity of the arguments and the interpretation of findings presented by Smillie (1922), who concluded that hookworms are slowly acquired and slowly lost. Chandler contends that hookworms are rapidly acquired and rapidly lost. He presents epidemiological data giving the results of comparison of egg counts on 915 persons imprisoned for varying periods of time in Calcutta, and points out that these results indicate that, in the absence of reinfestation, 50 per cent of the worms are lost within three months; 60 per cent within six months; and 70 per cent within twelve months. He stresses the importance of studying (1) groups of individuals received into an institution where reinfestation is not possible, and (2) normal communities at different seasons, by counting ova from the same individuals at frequent intervals.

If Chandler's theories are correct, then our policy of mass treatment of hookworm disease is at fault and must be revised. If hookworms are rapidly acquired and rapidly lost, we must rely solely upon sanitation and education as measures of control, and mass treatment must be abandoned, since it would not yield results proportionate to the effort and money expended.

From a recent study of infestation in a Panamanian village during the wet season, Cort and his associates (1929) conclude that in this particular group, with infestation approximately ten times that of Chandler's series, the level of infestation does not fluctuate markedly

* From the Field Research Laboratory of the International Health Division, Rockefeller Foundation, at Andalusia, Alabama.

between the wet and dry season and that no proportionally rapid loss of hookworms occurs. Sarles (1929), on the other hand, in his study on dogs, noted an analogy with Chandler's findings in his own observations of the rate of loss of *A. caninum*. The dogs lost most of their worms within six months, although a few worms persisted for twenty-one months.

### *Group Conditions and Methods*

We present here a study of the rate of loss of hookworms in three groups: (A) an institutional group in which reinfestation is not possible; (B) a rural community during a period when conditions are unfavorable for reinfestation; and (C) an individual who was infested in laboratory experimentation four years ago and who has had no subsequent infestation.

*A. Institutional Group.*—The first group comprises 78 children and young adults, ranging in age from 7 to 19 years, from the Cherokee Indian School, Cherokee, North Carolina. Supplementing this group we studied a small group of five young adult attendants at a hospital for the insane in southern Alabama.

The Cherokee Indian school children form an admirable group for study in accordance with Chandler's first suggestion. The children are at home during the summer months; from mid-September until June, they live at the school. The conditions in their homes in summer are favorable for the acquisition of larvae. The Indian homes are almost invariably situated close to a mountain stream; the majority are isolated dwellings on the mountain slopes. The soil is a sandy loam mixed with humus. The rainfall in the mountain area in the months from June through August is abundant, with a mean of 15.05 inches; and the summer temperatures (mean 72.0°F.) are favorable to hookworm larvae. Latrines are absent. The Indian children go barefoot about the home, as do their parents. The adults and older children frequent defecation sites along the banks of the streams somewhat removed from the dwellings, and at times use the stream itself. The younger children are more promiscuous in their choice of locations. They prefer sites about the dwellings, usually behind or in corners of the building, or by a clump of bushes near at hand. The moist shade of these defecation sites provides good conditions for the development and survival of hookworm larvae. Torrential showers sometimes wash pollution into the stream. The short period of time during which the school children can acquire larvae serves to limit the level of infestation. This fact is revealed by the low average intensity of infestation of the group studied. However, the presence of a few heavily infested persons in the group indicates that, in some instances, all factors favorable for infestation have coexisted.

The children's living conditions during the school months render further infestation practically impossible. The school is supplied with flush toilets, and personal observation has indicated that the children customarily use them. Furthermore, the children are provided with shoes upon entrance to the school, and atmospheric temperatures during the school months are unfavorable for development of hookworm larvae. Killing frosts may be expected from the middle of October until late April and even May, and winter temperature records of zero or slightly below are frequent. During the school year the children return home twice: first, for a few days late in October during the Indian Fair, and again at Christmas. In these visits to their homes, the time interval is too brief for significant acquisition of hookworm larvae under any conditions; and furthermore, the children are shod and atmospheric temperatures are low.

The Cherokee group was first examined two days after the opening of school, September 12, so that an accurate initial index of their infestation might be obtained. Preliminary examination by the salt-flotation method was made of specimens from the majority of the children in attendance (approximately fifty of the smaller boys were missed). The results indicated that, of 304 children, 170, or 56 per cent, had hookworms. This approximates closely the index found three years previously when 58.9 per cent of 341 children from the same institution harbored hookworms. This suggests that under identical conditions the incidence of infestation of the same community group tends to maintain equilibrium. The intensity of infestation in those found positive by the salt-flotation method was determined by the anti-formin-sugar method (Caldwell and Caldwell, 1926). As would be expected in a region where the season favorable for acquisition of hookworms is very short, not a few of the children were found positive by the salt-flotation method only, with egg counts very low or zero. These were discarded as of little value for the purpose of our study. Of the 170 positives, 41 boys and 41 girls were at first selected for study. Of these, one of the girls and eight of the boys were subsequently dropped from statistical consideration with the group, since they submitted specimens for from one to four months only.

From the group chosen, fecal specimens were collected for three days, consecutive if possible. Egg counts on this first series of specimens were made at the school. Subsequent collections of three-day specimens were made at monthly intervals under the direction of the supervisors of the boys and girls and were shipped parcel post, special delivery, to the Andalusia laboratory for egg counting. Collections were submitted throughout the school session from the middle of September through the middle of May. We received excellent cooperation, and wish here to express our thanks to the Bureau of Indian

Affairs for permission to carry forward this study; we wish also to acknowledge our great indebtedness to Mr. L. W. Page, Superintendent of the Cherokee Indian School, and to the supervisors of the school, for their cordial aid.

Despite the fact that the diet was practically the same for all the children, there was a good deal of variation among individuals in consistency and texture of stools. There were also day-to-day variations in stools from the same individual. All stools were translated to "formed basis," but this does not offer any exact standard. Egg counts at best are relative. The stools were graded by the same person and divided into the following classes: (a) firm, (b) formed, (c) soft formed, (d) soft to soft formed, (e) soft, and (f) diarrhetic. The latter was rarely encountered and, in the main, discarded. Stools which appear formed are not infrequently diluted with vegetable fibers, seeds, kernels, etc., so that soft and soft-formed stools, because of their uniform texture, may offer a higher count. In the winter months, with a higher proportion of protein in the diet, the stools were more uniform than in the autumn and spring months, when they contained fruit and vegetable pulp. The average of a three-days' collection aided in equalizing individual variations. Such three-day collections also offered valuable clues in detecting errors in submission of material, either inadvertent or deliberate.

The Cherokee girls offered unusually satisfactory material. In four of the nine months of collection all individuals submitted specimens; in the other months only one to three individuals failed to return specimens. In all, only eight specimens were missing in 360 different collections. More than 82 per cent of the collections represent three specimens each, and 94 per cent at least two. Close scrutiny of other parasite ova, character of the stools, and consecutive egg counts, indicated whether or not the stools submitted belonged to the individuals named. Only five specimens were discarded as apparently not *bona fide*. A few others had to be discarded because the amount submitted was insufficient.

The material from the Cherokee boys was not quite so satisfactory. Mixing or cheating was evident in some cases, and specimens were not so consistently returned each month. In February, the count from only fourteen of the thirty-three boys was obtained, because the names and dates were not recorded on some of the tins. In the other eight months, however, specimens were received from all but twelve individuals, six of the missing collections belonging to the month of May. Sixty-six per cent of the collections represents all three specimens; 90 per cent at least two. That is, of the total Cherokee group, by far

the greater proportion submitted all three specimens monthly and more than 90 per cent returned two each.

*Searcy Hospital:* As a supplement to the Cherokee group, we studied a group of five white attendants, from 18 to 21 years of age, from the Searcy Colored Insane Hospital. The collections, which were made at monthly intervals from October or November to April or May, were trustworthy, and in all cases counts were based on three specimens each.

*B. Rural Community.*—The rural group, composed of 47 children and young adults ranging in age from 2 to 19 years, is representative of the groups in the poorer rural districts of the southern coastal plain of Alabama, a section which has been shown to be singularly favorable for the acquisition of heavy hookworm infestations (Smillie and Augustine, 1925). Children and youths were chosen, rather than adults, as representing that group which would most probably have acquired new crops of hookworms in the previous summer months. The group included the children of all families (with two exceptions) along two rural routes. It happened that in this community five of the families were related; hence visiting was frequent and opportunities for the children to acquire infestations were not confined to their own homes.

For the most part, the farms have a very sandy soil, and the economic status of the people is low. Only an occasional home is provided with a latrine. Even young adults go barefoot for the greater part of the summer; the young children invariably do so. Although the older children put on shoes at the opening of school, the majority of the younger children go barefoot until cold weather sets in. Augustine (1926) indicated that in southern Alabama hookworm larvae were not present in great numbers in the soil after the first heavy frost, and that conditions were unfavorable for development or prolongation of life of the larvae when the mininum temperature approximated 50.0°F., or during periods of dry weather. Conditions affecting hookworm infestation in rural southern Alabama vary, therefore, from year to year, depending upon the temperature and precipitation in the autumn and in the early spring months. In the summer months (June through September) preceding our study, the conditions for the acquisition of larvae had been unusually favorable: temperatures were high and there was more than normal rainfall, as is shown in the tabulation below.

| | June | July | Aug. | Sept. | Mean |
|---|---|---|---|---|---|
| Maximum temperature | 88.8 | 91.2 | 93.9 | 87.2 | 90.3 |
| Minimum temperature | 69.0 | 70.8 | 71.5 | 67.6 | 69.8 |
| Precipitation | 6.25 | 5.0 | 4.37 | 6.0 | 5.4 |

Although, on the whole, the October temperatures were high, a three-week drought followed by a rain and a cold snap, rendered conditions detrimental for hookworm larvae during the greater part of the month. During the last few days of October and the first week of November, weather conditions, although not optimum, were not detrimental, showing a mean temperature of 69.7°F. and a precipitation of 4 inches. However, from the middle of November until late spring, conditions for the acquisition of larvae may be considered unfavorable. From that date until the last week in February, the temperatures were low, with an average minimum of 41.8°F., and there were several prolonged cold snaps, with temperatures as low as 19.0°F. Temperatures were higher during the latter part of February, but the weather was unusually dry. The month of March was unnaturally cold for early spring (average maximum temperature 66.2°, average minimum, 40.3°) and in April there was a prolonged drought. Such acquisitions of larvae as might have been possible during April would not be registered until May, so that the late April egg counts were considered the final counts for the study of rate of loss in this particular group.

Collections for three consecutive days were obtained by personal visits to each home at monthly intervals from late October until late April. The April collections were less inclusive as a group (specimens of 11 individuals missing) because of moving or absence of some of the individuals. In the other six monthly collections none were missing in three months and a total of eight only in the other three months. Almost 84 per cent of the specimens submitted represented three days' collection each; over 97 per cent, at least two each.

*C. One Individual for Four Consecutive Years Following Infestation.*—The third part of the study includes counts from one individual only. Chandler questions Smillie's deduction of a slow rate of loss as represented by four individuals who, after three or four years of absence from field labor, still harbored worms in numbers comparable to the average worm index of laborers in the field, on the ground that these individuals may have become infested from their own stools. Such question cannot fairly be raised in the case of X, of our own laboratory force. X acquired infestation in the summer of 1926 during the course of experimental work in the daily isolation of hookworm larvae by the Baerman method. Because of difficulty in adjusting the clamp, the centrifuge tube containing larvae overflowed, and infestation occurred. Characteristic blisters formed between the middle and index fingers. These were few in number, and itching was not pronounced. The work in which X was engaged was ended early in September, 1926, and during the subsequent four years, experimental

work with hookworm cultures was minimal. With the very few cultures handled by X in the interval, great care was exercised to avoid further infestation.

## RESULTS

Since our study embraces distinct groups, it may be of interest, in judging the significance of the results, to note their characteristic differences. The two groups, Cherokee and Alabama rural, represent two races, the one Indian, and the other descendants of Colonial English and Scotch ancestors. The Cherokee group is further characterized by infestations with Ascaris and Trichuris in a high proportion of cases, 69.8 per cent harboring Ascaris, 43.8 per cent Trichuris, and 30.1 per cent both parasites. The Alabama group has a pure hookworm infestation. The economic status and personal living habits of the two groups while at home are similar. The Cherokee group, however, because of its residence in an institution three-fourths of the year, shows

TABLE 1.—*Intensity Groups: Egg-Count per .01 Gm., Initial Survey*

| Cherokee | | | | | | | Alabama | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Groups (Ova per 0.01 Gm.) | Girls | | Boys | | Total | | Groups (Ova per 0.01 Gm.) | Girls | | Boys | | Total | |
| | No. | Per Cent | No. | Per Cent | No. | Per Cent | | No. | Per Cent | No. | Per Cent | No. | Per Cent |
| 1– 9 | 16 | 40.0 | 8 | 24.2 | 24 | 32.9 | 1– 9 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10–20 | 12 | 30.0 | 7 | 21.2 | 19 | 26.0 | 10– 20 | 5 | 10.6 | 3 | 11.1 | 2 | 10.0 |
| 21–50 | 7 | 17.5 | 14 | 42.4 | 21 | 28.8 | 21– 50 | 15 | 31.9 | 5 | 18.5 | 10 | 50.0 |
| Over 51 | 5 | 12.5 | 4 | 12.2 | 9 | 12.3 | 51–150 | 16 | 34.0 | 11 | 41.8 | 5 | 25.0 |
| | | | | | | | Over 151 | 11 | 23.5 | 8 | 29.6 | 3 | 15.0 |

the low degree of infestation characteristic of short periods of acquisition. As is seen in Table 1, 87.0 per cent of the Indian children have egg counts of less than 5,100 per gram; almost one-third have less than 1,000; the mean egg count approximates 2,800. In the Alabama group, representing a long period favorable for acquisition, none have counts of less than 1,000; 51.0 per cent have over 5,100 eggs per gram, and 24.0 per cent have 15,100 eggs per gram. Their mean count is 9,500, more than three times that of the Cherokee group.

In both groups the males are more heavily infested than the females: 70.0 per cent of the Cherokee girls have counts of less than 2,100, while 55.0 per cent of the boys have counts above that number. Similarly, in the Alabama group, although the children come from the same families, 60.0 per cent of the girls have counts of less than 5,100, whereas 71.0 per cent of the boys have counts greater than 5,100.

In general the groups comprise the same age classification (Table 2), but the Cherokee group includes only two children of less than 8 years of age, so that only two age classifications are considered for

that unit. Among the Cherokee children, those of the younger age group have a much heavier infestation than the older children. Although the youngest children of the Alabama group have a slightly higher percentage (14.2) in the lowest intensity group and slightly less (14.3 per cent) in the highest intensity group, 57.0 per cent of the very young children have egg counts of between 5,100 and 15,100. However, since the stool size (Stoll and Sweet, 1929) of younger children is less, the counts per gram probably do not represent a worm burden equivalent to that accompanying comparable egg counts in the other groups. The difference between the intensity of infestation of the younger children (8-14 years) and the older children (15 years and over) is too slight to be significant, although it suggests a somewhat lower infestation in the highest age group.

TABLE 2.—*Intensity of Hookworm Infestation, by Age Groups*

| | Cherokee | | | | Alabama | | | |
|---|---|---|---|---|---|---|---|---|
| | Age in Years | | | | Age in Years | | | |
| Groups | Less Than 8 | 8-14 | 15+ | Groups | Less Than 8 | 8-14 | 15+ |
| No. studied..... | 2 | 50 | 23 | No. studied..... | 14 | 24 | 19 |
| Ova per 0.01 Gm. | | | | Ova per 0.01 Gm. | | | |
| 1- 9..... | .. | 26.0 | 47.8 | 1- 9..... | 0 | 0 | 0 |
| 10-20..... | 100.0 | 32.0 | 13.0 | 10- 20..... | 14.2 | 8.3 | 11.2 |
| 21-50..... | .. | 28.0 | 30.4 | 21- 50..... | 21.5 | 37.5 | 33.3 |
| Over 51..... | .. | 14.0 | 8.7 | 51-150..... | 50.0 | 29.2 | 33.3 |
| | | | | Over 151..... | 14.3 | 25.0 | 22.2 |

In Table 3 are tabulated the average egg counts for each monthly examination of the Cherokee and Alabama rural group. Since a few heavy infestations have undue weight in calculating the arithmetic mean, we have calculated the geometric mean, as a more accurate basis for comparison; and since stool specimens of some individuals were missing in certain months, the initial mean counts of the same individuals are also given and the variations expressed as percentages of the initial mean. Table 3 clearly indicates no rapid loss of worms, as evidenced by egg counts in a period of nine months in the Cherokee group and seven months in the Alabama group. The trend, in fact, is upward. This is shown graphically in the chart on page —. There are fluctuations in both, and the rise in the Cherokee group may not be significant. The variations may be due to inherent difficulties in egg-counting methods, and particularly to differences in stool character. As suggested previously, in the winter months a greater proportion of the stools were more uniformly textured and showed higher counts. Since the rise is maintained, however, it may indicate increased ova production of young females acquired in the summer.

In the Alabama group the increase in egg counts is greater and is probably significant, indicating oviposition by females acquired in the summer and autumn months. We have, in fact, concrete evidence of oviposition of newly acquired females in the case of one boy who had a severe ground itch late in October. His initial count, October 19-22, was approximately 4,000; this was maintained in November, but it was more than doubled in December, and subsequent counts continued on a high level. It seemed probable from other counts that the same situation was true of several individuals of the group. The high point in the curve is undoubtedly an aberrant high, and a smoothing of the curve would suggest a general increase of 10 to 20 per cent.

TABLE 3.—*Variations in Mean Monthly Egg Counts per Gram (Formed Basis) of Cherokee and Rural Groups*

| Month | Number Cases | Monthly Geometric Mean | Initial Geometric Mean | Per Cent of Initial Mean |
|---|---|---|---|---|
| A. Total Cherokee Group | | | | |
| September | 73 | 1644 | 1644 | 100.0 |
| October | 71 | 1641 | 1644 | 99.8 |
| November | 73 | 1676 | 1644 | 101.9 |
| December | 71 | 1667 | 1625 | 102.6 |
| January | 71 | 1910 | 1645 | 116.1 |
| February | 53 | 1924 | 1597 | 120.5 |
| March | 68 | 1594 | 1599 | 99.6 |
| April | 73 | 1876 | 1644 | 114.2 |
| May | 64 | 1692 | 1641 | 103.1 |
| B. Alabama Rural Group | | | | |
| October | 47 | 6480 | 6480 | 100.0 |
| November | 45 | 6010 | 6581 | 91.3 |
| December | 47 | 7205 | 6480 | 111.2 |
| January | 47 | 9145 | 6480 | 141.1 |
| February | 44 | 7249 | 6474 | 111.9 |
| March | 44 | 8175 | 6509 | 125.6 |
| April | 36 | 6235 | 5530 | 112.7 |

The high point represents not only the peak of egg production of newly acquired females but a coincidence of high counts from several individuals whose counts otherwise indicate stable equilibrium. We noted here, as in the Cherokee group, that among all members of certain families there occurred at this time dense, finely textured stools, probably the result of diet and habits in cold weather. The rural group is not large (47), so that fluctuations of this type would raise the percentages abnormally.

We have analyzed our data further by dividing the individuals into three groups: (1) those showing a definite loss; (2) those showing definite increase; and (3) those showing stability. Table 4 gives the number and percentages of cases in these groups, and the mean of the initial counts compared with the mean of the total counts for the individuals of each group. From 12 to 14 per cent of both groups

show a decided loss, more definitely significant in the Alabama group than in the Cherokee unit. A fair proportion of the individuals show a definite increase: 38.3 per cent in the Alabama group and 21.9 per cent in the Cherokee group. The counts of approximately 47.0 per cent of the individuals in the Alabama group and of 66.0 per cent of those in the Cherokee are stable. Of the individuals showing loss, the mean count is reduced by approximately 30 to 40 per cent from the initial count, whereas the mean gain is from 35 to 45 per cent higher than the initial count. A rapid loss of hookworms does not occur either in the group as a whole or in individuals of the group.

*Influence of Intensity of Infestation on Rate of Loss of Worms*

We have further divided the groups in relation to intensity of infestation (see Table 5). Because of the small number involved when the unit is divided into intensity classifications, the mean count for

TABLE 4.—*Comparison of Mean Average Count with Initial Mean Count of Groups, Showing (1) Loss, (2) Gain, and (3) Equilibrium*

| Group | No. | Per Cent Total | Initial Geometric Mean | Average Geometric Mean | Per Cent of Initial Mean |
|---|---|---|---|---|---|
| | | Alabama Rural Group | | | |
| Loss | 7 | 14.9 | 6519 | 3830 | 58.7 |
| Gain | 18 | 38.3 | 5228 | 7206 | 137.7 |
| Equilibrium | 22 | 46.8 | 7707 | 7725 | 100.2 |
| | | Cherokee Group | | | |
| Loss | 9 | 12.3 | 1454 | 1031 | 70.9 |
| Gain | 16 | 21.9 | 2666 | 3884 | 145.7 |
| Equilibrium | 48 | 65.8 | 1393 | 1476 | 105.9 |

three months in the Cherokee group and for two months in the Alabama group have been tabulated. The low intensity group of the Cherokee school is of interest in comparison with Chandler's prison group, which had a light infestation upon entrance to the prison. In this group a consistent but not significant loss from the initial count is suggested (less than 10.0 per cent). The second intensity group (10-20 ova per .01 gram of feces) shows a consistent increase. This is true also of the Alabama group, which showed an intensity of 10-50 ova per .01 gram of feces. In the other group in both units, the variations are not significant.

*Relation of Age to Loss of Hookworms (Alabama Rural Group)*

We have divided the children in the Alabama rural unit into three age groups: (a) under 8 years; (b) 8-14 years; (c) 15 years and over (see Table 6). In comparison with the initial egg count, the youngest age group shows a consistent increase; the older age group shows

TABLE 5.—*Mean (Geometric) Egg Count per Gram (Formed Basis) of Intensity Groups in Periods Stated*

| Intensity Groups (Ova per 0.01 Gm.) | No. in Initial Group | Time Interval | Total Number | Mean for Whole Period | Initial Mean | Per Cent |
|---|---|---|---|---|---|---|
| | | Cherokee Group | | | | |
| 1- 9 | 24 | Sept.-Nov. | 72 | 438 | 471 | 93.2 |
| | | Dec.-Feb. | 67 | 466 | 486 | 95.7 |
| | | Mar.-May | 67 | 437 | 485 | 90.1 |
| 10-20 | 19 | Sept.-Nov. | 55 | 1735 | 1601 | 108.4 |
| | | Dec.-Feb. | 49 | 2195 | 1575 | 139.3 |
| | | Mar.-May | 55 | 2303 | 1588 | 145.0 |
| 21-50 | 21 | Sept.-Nov. | 63 | 3253 | 3269 | 99.5 |
| | | Dec.-Feb. | 53 | 3339 | 3180 | 104.7 |
| | | Mar.-May | 60 | 3089 | 3258 | 94.8 |
| 51 plus | 9 | Sept.-Nov. | 27 | 10660 | 9802 | 108.7 |
| | | Dec.-Feb. | 26 | 12510 | 9730 | 128.5 |
| | | Mar.-May | 25 | 10140 | 9688 | 104.6 |
| | | Alabama Rural Group | | | | |
| 10- 50 | 20 | Oct.-Nov. | 39 | 2545 | 2611 | 97.5 |
| | | Dec.-Jan. | 40 | 3741 | 2606 | 143.5 |
| | | Feb.-Mar. | 38 | 3409 | 2613 | 130.4 |
| | | Mar.-Apr. | 37 | 3429 | 2594 | 132.2 |
| 51-150 | 16 | Oct.-Nov. | 31 | 8857 | 9322 | 95.0 |
| | | Dec.-Jan. | 32 | 10038 | 9295 | 108.0 |
| | | Feb.-Mar. | 29 | 10434 | 9433 | 110.6 |
| | | Mar.-Apr. | 26 | 10042 | 9225 | 108.8 |
| 151 plus | 11 | Oct.-Nov. | 22 | 18750 | 20094 | 93.3 |
| | | Dec.-Jan. | 22 | 24380 | 20094 | 121.3 |
| | | Feb.-Mar. | 21 | 22080 | 20140 | 109.2 |
| | | Mar.-Apr. | 17 | 21060 | 20060 | 105.0 |

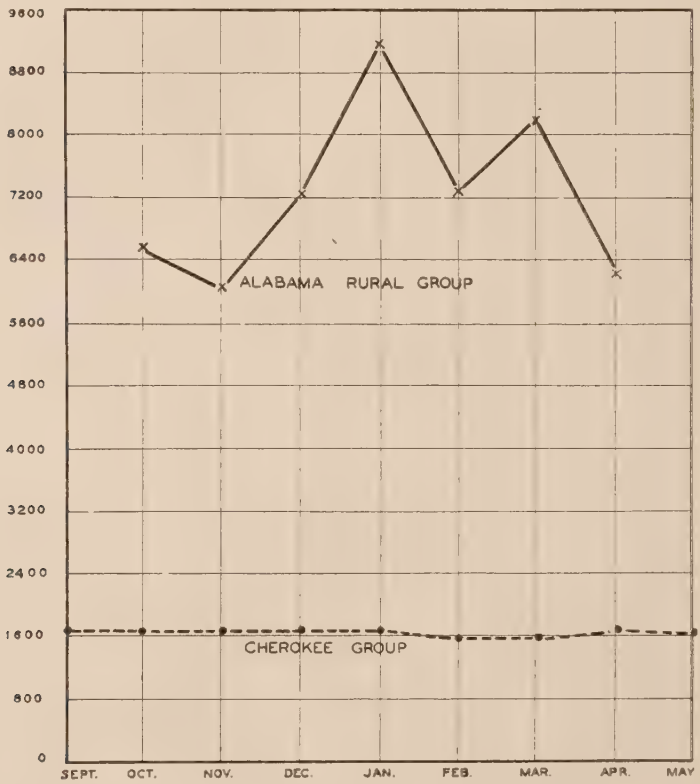
TABLE 6.—*Mean Monthly Egg Counts per Gram (Formed Basis), by Age Groups (Rural)*

| Month | Number of Cases | Monthly Geometric Mean | Initial Geometric Mean | Per Cent of Initial Mean |
|---|---|---|---|---|
| | | Less Than 8 Years | | |
| October | 14 | 5224 | 5224 | 100.0 |
| November | 13 | 4744 | 5033 | 94.2 |
| December | 14 | 7613 | 5224 | 145.7 |
| January | 14 | 8739 | 5224 | 167.2 |
| February | 13 | 7676 | 5225 | 146.9 |
| March | 12 | 8908 | 5608 | 158.8 |
| April | 12 | 6060 | 4527 | 133.8 |
| | | 8–14 Years | | |
| October | 24 | 7337 | 7337 | 100.0 |
| November | 23 | 7005 | 7700 | 91.0 |
| December | 23 | 7466 | 7337 | 101.7 |
| January | 24 | 10210 | 7337 | 139.1 |
| February | 22 | 7955 | 7045 | 112.9 |
| March | 23 | 8727 | 6957 | 125.4 |
| April | 18 | 6956 | 6484 | 107.2 |
| | | 15 Years and Over | | |
| October | 9 | 6506 | 6506 | 100.0 |
| November | 9 | 5716 | 6506 | 86.2 |
| December | 9 | 6014 | 6506 | 92.4 |
| January | 9 | 7321 | 6506 | 112.5 |
| February | 9 | 5316 | 6506 | 81.7 |
| March | 9 | 6169 | 6506 | 94.8 |
| April | 6 | 4755 | 5119 | 93.3 |

a consistent loss. This suggests that the youngest children had acquired more worms than the group next in age; that among children 15 years or older the loss of old worms exceeded any recent gain of new worms.

### *Results in Hospital Attendants*

The general results obtained from the Alabama and Cherokee groups are confirmed by the findings among the attendants of the Searcy




Monthly Variation in Hookworm Egg Count from Initial Mean (Geometric).

Colored Insane Hospital. Because of the small number in this group and the variation in intensity within it, the averages are not computed. The examinations over a period of from seven to eight months in this group of five young adults of 18 to 21 years of age, show that one attendant, lightly infested, whose subsequent egg counts show an increase over the initial count, had probably acquired hookworms shortly before entrance, and that the other four, including one light, one moderate, and two heavy infestations, maintained undoubted stability during the entire period.

### *Results of the Study of Hookworm Intensity of One Individual Over a Period of Four Years*

In the two groups discussed above, a rapid loss of worms is not apparent within seven to nine months. With this finding we may compare the intensity of infestation of one individual (laboratory worker X) over a period of four years. X was infested in August, 1926. A series of counts in October, 1926, averaged 1,400 ova per gram of feces. Six months later the average of the series was approximately 1,800 ova per gram of feces, indicating either an increased ova production of young maturing females or variations due to stool character. A year later the egg count also approximated 1,800. In 1929 and 1930 egg counts were made at approximately monthly intervals. There was a good deal of individual variation in counts, ranging from 1,200 ova per gram in soft formed stools or stools with roughage, to 3,500 ova in hard stools with delayed peristalsis. The average has

TABLE 7.—*Variations in Egg Counts per Gram (Formed Basis) of X During a Four-Year Period*

| 1926 | 1927 | 1927 | 1929 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Oct. | April-May | 5/28-29 | 3/21 | 5/14-16 | 5/22-24 | 6/19-26 | 7/24-27 | 8/8 | 10/30 | 11/13 | 12/6 |
| 1400 | 1800 | 1750 | 1700 | 2150 | 1820 | 2150 | 2700 | 1990 | 1830 | 1800 | 1850 |

| 1930 | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1/30-31 | 2/1-2 | 2/12 | 5/13-17 | 5/26-30 | 7/18-30 | 7/28-29 | 8/1-14* | 9/19-22 | 9/27 | 10/30 |
| 2100 | 1860 | 1600 | 1890 | 1440 | 2530 | 2350 | 1600 | 1700 | 1400 | 2100 |

* Average of fourteen consecutive counts.

approximated 1,800 ova, as shown in the tabulated results (Table 7). This case offers convincing evidence that, in one individual, hookworms may establish themselves and oviposit for a period of four years without apparent loss in numbers.

Since X has a light infestation, it is not likely that there is an actual loss of worms with compensation in egg count due to increased ova production of remaining females. We believe that our results show that hookworms acquired by human beings at any one time or season, after establishing themselves, may have a definite life span, which may be four years or longer, certainly much longer than the life span found for *A. caninum* by Sarles (1929).

## SUMMARY

The rate of loss of hookworms was studied by the egg-count method. Counts were made at monthly intervals on the fecal specimens of individuals in three different groups: (1) an institutional group (a) of 73 children between the ages of 7 and 19, from the Cherokee Indian

School, examined over a period of nine months, supplemented by (b) a group of five young adult attendants in a hospital in Alabama, studied over a period of eight months; (2) a rural community in southern Alabama, comprising 47 children of from 2 to 19 years of age, examined over a period of seven months. Reinfestation was not possible at the Cherokee school during the period of study, and conditions for acquisition of hookworms were highly unfavorable in the Alabama community during the period of examination. In both groups, conditions for the acquisition of the worms had been favorable in the period preceding our study. (3) One individual who had acquired experimental infestation in 1926, with no possibility of reinfestation, was studied for a period of four years.

There was no apparent loss in worm burden during four years, in the one individual; and in the groups, although a few individuals lost worms, the hookworm intensity of the majority remained stable. In both groups some individuals showed an increase in egg counts, which probably was due to maturing and ovipositing on the part of the females recently acquired.

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# A GLIMPSE INTO THE LIFE HISTORY OF THE TAPEWORM OF SHEEP, *MONIEZIA EXPANSA*

D. F. Sinitsin
WASHINGTON, D. C.

To date there has been nothing known of the life history of *Moniezia expansa*. In the present paper, I give an account of my studies on the eggs which show quite distinctly the way sheep become infested with this parasite. My observations begin from the moment when eggs, enclosed in the body of ripe segments, leave the intestine of the sheep with the excrements and ends at the forty-fifth day of their being at large and outside of any host.

The main features of an egg, taken from a ripe segment, are shown in figure 1. The shape is nearly that of a regular hexahedron, with all its angles rounded off. The shell is composed of three envelopes or coats: the exterior (*h*, Fig. 1) is hard and non-elastic, horny and indissoluble in the digestive fluids though permeable to water; the interior (*i*) is soft and of a fibrillar structure; the intermediate (*o*) consists of small drops of an oily substance, and is the thickest of all. The horny coat meets the fibrillar at the angles of the egg, so that the oily coat is distributed only on the sides of the hexahedron, being absent at the angles.

Inside the shell, there is lying loosely a structure, known under the name of the pyriform, or pear-shaped apparatus; this will be called the capsule. In the capsule can be distinguished two portions: the body, which is spherical, and the horns, which are cylindrical, gradually tapering to their ends. The substance of the capsule is homogeneous, perfectly translucent, elastic, permeable to water and dissoluble in the digestive fluids, including the saliva, and, under certain conditions, in mucus of respiratory cavities. The walls of the capsule grow thinner toward the top of the capsule, being thickest at its base where the pair of horns arise. Inside, the body of the capsule is divided into two compartments: the upper, where the embryo is enclosed (*e*) and the lower that contains an apparatus for feeding the horns (*s, f*). Both horns are of equal size and shape. They are slightly bowed and bear a common cap (*c*) at their distal ends. The feeding apparatus of the horns consists of two protoplasmic and nodular strings, one for each horn, and of two granular cells, which belong only to one of the horns. The strings are attached to the ceiling of the lower compartment and stretch along the canals of the horns up to their ends.

The embryo itself (*e*) is enclosed in a membrane (*m*), one part of which is closely attached to the capsule, while the other is loose and makes the partition between the upper and lower compartments. The partition bears two funnel-shaped pits opposite the horns; the smaller is directed toward the horn which is furnished with the feeding or trophic cells, the larger is opposite the horn without trophic cells. The body of the embryo is not connected with its membrane, under which it can move quite freely. It is composed of about twelve cells, arranged in accordance with bilateral symmetry, and is armed with three pairs of strong hooks.

Of all the parts just described the shell looks the most promising in regard to possible conclusions about the immediate future of the egg. In fact, an oily substance, enclosed between two coats permeable to water, should render them waterproof and make the eggs capable of withstanding dryness. In order to verify this conclusion I have set up a series of experiments on this point. The experiments proved the shell to be a very efficient protection against desiccation. During forty-five days some portions of the eggs were several times dried up to a state shown in the photograph (text figure), and in spite of that they did not lose their vitality. The mechanism which prevents complete drying out works in this way. As soon as an egg gets in touch with the air, it promptly loses a part of its water and consequently acquires the shape of a hexahedron with facets deeply bent toward the center (Fig. 4). If one makes an imaginary section through such an egg (Fig. 5), then it appears why an egg in such a state stops losing water: because the oily layer of the shell under these conditions turns into almost a continuous layer which prevents the drying up of the soft contents of the egg, whereas the parts permeable for water shrink into a lamella but slightly connected with the cavity of the egg.

The fact of the existence of such a complicated apparatus, which can be brought into action many times without losing its efficiency, draws one to the conclusion that under normal conditions in nature the eggs of *M. expansa* do not reach their prospective host at once, but are destined to be at large rather a long time. On the other hand, as under natural conditions manure in mass appears to be more frequently moist than dry, it is reasonable to expect that during these periods the eggs do not stop development, which was perhaps overlooked by the former investigators. Moved by these considerations, I put a number of ripe segments into a moist chamber and kept them there forty-five days, every day examining the condition of the eggs in the experiment. I was not disappointed in my expectations: the eggs continued to develop and change, revealing at the same time some peculiari-

ties that distinctly hinted at events the eggs were to pass through in the next phase.

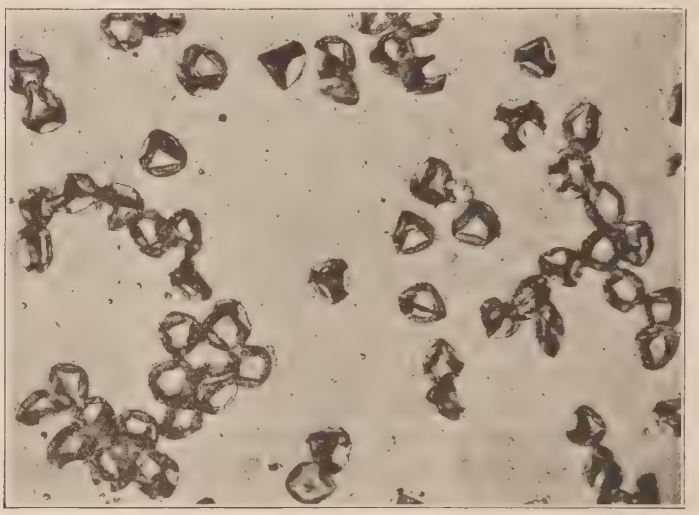
The changes in the capsule appear to be most instructive for an observer. Being at first movable inside the shell, the capsule soon becomes attached to it by the cap covering the ends of its horns. If at this time of development the shell happens to be destroyed, the capsule frees itself with parts of the fibrillar coat of the shell attached to its cap. This process of growing into the shell is going on through all the forty-five days of the experiment. At the same time the cap gradually acquires the shape of a disc, later on, of a disc with a hollow in its center and at the forty-fifth day it is a thick ring firmly grown into the horny coat of the shell (*c*, Figs. 2, 3). But still more significant changes occur in the horns of the capsule. As has been already mentioned, one of the horns is furnished with two nourishing cells, while another has only remnants of such attached to the proximal end of the protoplasmic string. In accordance with this only one of the horns continues to grow in length, while the other does not. This brings about the rotation of the body of the capsule and in consequence the twisting of the longer horn about the shorter one. At the forty-fifth day the greater part of the eggs show their capsules turned at an angle a little more than 90° (Fig. 2), but some were turned to the full 180° (Fig. 3).

Of other changes the following are worth mentioning: The shell becomes very fragile. The slightest touch breaks it in pieces and frees the capsule with its twisted horns. This should be put into logical connection with the appearance of a permanent communication between the cavity of the shell and the outside world through the opening in the center of the ring, which meantime abolishes the main function of the shell as a protective mechanism against dryness. As for the embryo itself, during this period it develops a pair of large glands (*g*, Fig. 2) that open at the base of the hooks; besides, it becomes more responsive to the changes in external conditions. If a capsule with such an embryo is put into a neutral fluid like the nasal mucus, for instance, the embryo begins to move, working, at the same time, energetically with its hooks. I had no chance to study eggs that have reached the end of their development, therefore I never saw an embryo finish its work successfully and free itself from both the membrane and capsule. Possibly this may not occur earlier than the sixtieth day of development in moist feces.

The biological meaning of all that happens to an egg during forty-five days of its devolpment is quite clear. The shell has not to protect the embryo which is well protected by the capsule, but it protects

the capsule itself, while it is soft and has not yet reached the end of its development. When this is accomplished and the horns have formed a boring apparatus, there is no reason for the longer existence of the shell, therefore it becomes very fragile, particularly when dried. Thus desiccation in the end of this period appears to be a necessary condition of development. If such an egg happens to be destroyed, it frees the capsule which breaks off where it is thinnest, that is, at the points of its connection with the ring, and turns into a particle of dust.

What happens next, easy to guess. The horns keep on twisting while they are dry, but as soon as they get in touch with a moist object,



Text Figure.—A microphotograph of the eggs dried up on the slide glass. Magnified 107 times.

they begin to untwist, the thickened part of the capsule between the horn bases acting as a spring. If it happens that this moist object is the soft tissue of an animal, and the end of the gimlet is resting on this surface, then the boring apparatus will be set into action and eventually the capsule becomes attached. Such conditions may be found only in the respiratory organs of mammals, that is, in the nasal cavities and in the cavities of trachea, bronchi and alveoli, possibly also on the conjunctiva. On the basis of such conjectures I devoted particular attention to the reaction of both capsula and embryo to the mucus from these organs and found that the embryos move most energetically in the mucus of the trachea and, what is of a great importance, this mucus dissolves the capsule in less than thirty minutes.

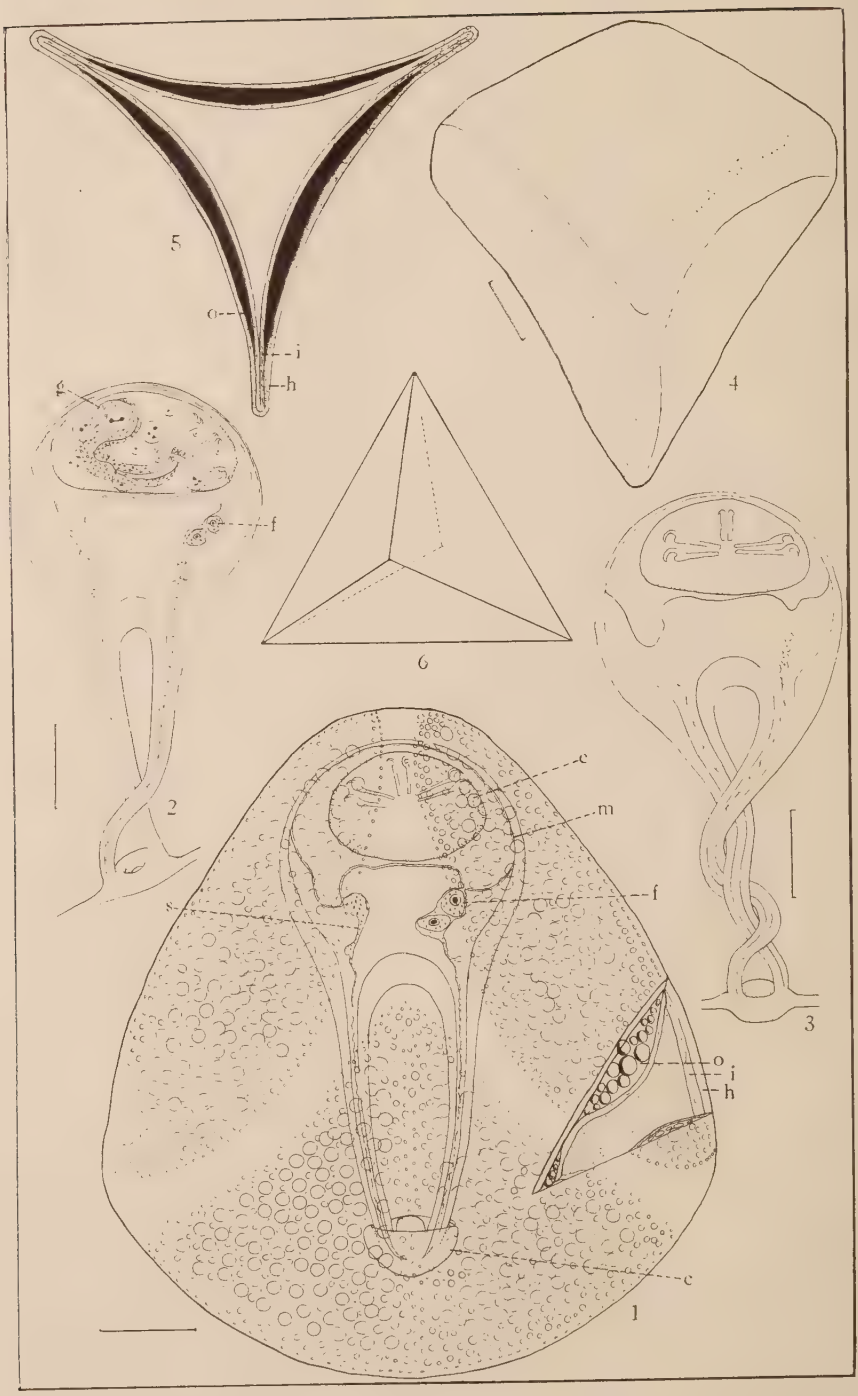



PLATE XXI

Thus one may conclude that in the life history of *Moniezia expansa* an intermediate host is lacking. The embryo, after a period of about two months development in moist materials, returns again to the sheep by the air. Perhaps it enters the capillaries of the windpipe or alveoli and, by the aid of the circulatory system, eventually reaches the intestine. If the host happens to be a pregnant female, it is quite possible that some of the embryos may reach the intestine of the fetus.

Independently of how near to the truth is the hypothesis advanced in the last paragraph, the fact remains that the embryo, before it has reached its prospective host, has to live at large at least two months, and during this time it is susceptible to measures directed to its destruction. From this point of view, the period when the embryo is at the end of its development and is ready for its airy trip, is the most critical for it, because if, at this time, it happens to be moistened without its host, life will be terminated.

## Explanation of Plate XXI

Fig. 1.—Egg of *M. expansa* from ripe segment. *c*, cap covering ends of capsule horns; *e*, the embryo; *h*, external or horny coat of shell; *f*, cells feeding the horn; *s*, protoplasmic string related to horn; *i*, internal or fibrillar coat of shell; *m*, membrane of embryo; *o*, intermediate or oily coat of shell.

Fig. 2.—Capsule at fortieth day of development. *f*, feeding cells of embryo; *g*, glands of embryo.

Fig. 3.—Capsule at forty-fifth day of development.

Fig. 4.—Egg in dried condition.

Fig. 5.—Optical section through dried egg.

Fig. 6.—Hexahedron, type of form of eggs of *Moniezia expansa*.

Scales for all figures represent 0.01 mm.

# NOTE ON SOME LARVAL NEMATODES FOUND IN FROGS *

A. C. Walton
Knox College, Galesburg, Ill.

In 1924 Steiner reported the presence of two species of larval ascarids in cysts in the stomach wall and liver of the Carolina Tree Frog (*Hyla carolinensis*) to which he gave the names *Agamascaris odontocephala* and *Agamascaris enopla*. He also added careful descriptions and figures for the use of future workers. Since that time the present writer has had the opportunity of examining a considerable amount of Amphibian material from various parts of the United States and has found that the form *Agamascaris odontocephala* is of rather wide distribution, both as to range and as to host species, as follows:

*Rana catesbiana,* obtained from South Carolina, Louisiana, Virginia, Illinois, and the District of Columbia, afforded specimens taken from the body cavity and from cysts in the stomach wall.

*Rana pipiens,* obtained from Nebraska, Michigan, Illinois, Iowa, and Maryland, furnished specimens taken from the body cavity and from cysts in the stomach wall, the intestinal wall, the bladder wall, and the liver.

*Rana clamitans,* obtained from Maryland and the District of Columbia, showed encysted specimens; and

*Rana sylvatica,* from Maryland, also furnished encysted examples.

The wide-spread occurrence of this larval form in several species of frogs and the absence of any adult forms in frogs which can be traced back to these larvae, indicates the probability of another host form of similar wide-spread distribution. The natural enemies of the frogs include various snakes and a number of species of birds, particularly of the heron and duck groups. Experimental feeding given to a number of ducklings raised from wild stock to serve as decoys for hunters along the Mississippi River were tried to see whether infestation could be passed on. In only one case were forms recovered which seemed at all related, and these were still immature encysted forms in the stomach wall which could not be distinguished from forms obtained from the frogs. The attempted infestation was either unsuccessful or else the mallard is not the natural host for this parasite. Further experiments, however, are in progress.

This record is presented in the hope that other investigators more favorably located may find greater success in recognizing the other host and of obtaining the adult form of this wide spread parasite of possible economic importance.

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* Contribution from the Biological Laboratories of Knox College, No. 37.

The greater number of specimens have permitted the establishment of a wider range of measurements than those reported by Steiner, but all forms agree perfectly in the details of body structure as reported in his paper. The excretory pore is located just caudad of the nerve ring.

Measurements: Total length, 17 to 54 mm.; greatest width 0.4 to 0.7 mm.; mouth-nerve ring distance, 0.26 to 0.45 mm.; length of esophagus, 1.4 to 3.5 mm.; and anus-tail distance, 0.1 to 0.25 mm.

Cysts in the stomach and intestinal walls of specimens of *Rana catesbiana* taken in northern Indiana afforded examples of a larval spirurid (physalopterid) which is of considerable interest in that among the one hundred recorded species of the genus Physaloptera, only one, *P. amphibia* v. Linst. 1899, has been reported from an Amphibian host (*Rana macrodon,* Luzon. P. I.).

The cysts, which measured from 0.15 to 0.2 mm. by 0.45 to 0.7 mm., contained larvae that showed, in spite of their immature condition, sufficient development of the lip structures to enable the identification of these forms as belonging to the genus Physaloptera and to an hitherto undescribed species. Von Linstow described his form as possessing lips, each armed with a large external and a small tripartite internal tooth. The present form, at least in the largest specimen, showed that the external tooth is large and cone-shaped while the internal tooth is small and bipartite. In addition there are two very small bipartite (?) lateral teeth and a minute row of denticles between each lateral tooth and the median internal tooth. The cuticula is slightly reflected over the lips. This type of dentition, if characteristically adult, definitely separates this material from that studied by Von Linstow, and places it nearer the group of forms represented by *P. quadrovaria* Leiper 1908.

The larval measurements are as follows: Total length, 1.5 to 4 mm.; greatest width, 0.055 to 0.09 mm.; length of muscular esophagus, 0.07 to 0.12 mm.; length of glandular esophagus, 0.5 to 0.24 mm.; position of nerve ring, 0.05 to 0.08 from anterior end of esophagus; anus-tail distance, 0.045 mm.

Type specimen: No. 12.211, Ward Collection, University of Illinois.

Type host: *Rana catesbiana.*

Type locality: Northern Indiana.

Because of the apparent difference from the only adult form recorded from Amphibian hosts, *P. amphibia*, these larvae are tentatively assigned the name of *Physaloptera ranae.* The generic term of Agamospirura for larval spirurids is not used since these larvae are unquestionably members of the genus Physaloptera, *s. s.*

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# SOCIETY PROCEEDINGS

## HELMINTHOLOGICAL SOCIETY OF WASHINGTON

*One Hundred Thirty-First to One Hundred Thirty-Fourth Meetings*

The one hundred thirty-first meeting was held September 20, 1930.

Dr. N. A. Cobb presented a note and demonstration on a commensalism between bacteria and free-living marine nematodes; also notes (1) on the penetration by Mermis of the enteron of the grasshopper, (2) on the mathematical formula of Tricoma, and (3) on the sperm cells of nemas.

*Ascaridia numidae,* a parasite of the guinea hen, *Numida meleagris,* in Louisiana, by Dr. G. Dikmans. This parasite was originally reported by Leiper under the name of *Heterakis numidae* from *Numida ptilorhyncha* from the White Nile and Uganda, Africa. Gendre in 1909 reported it from *Numida meleagris* from French Guinea under the name of *Heterakis calcarata.* Mönnig in 1923 reported it from *Numida papillosa transvaalensis.* He used the name *Ascaridia numidae* and placed both Leiper's name *Heterakis numidae* and Gendre's name *Heterakis calcarata* in synonomy. It was reported as a parasite of the guinea hen from Porto Rico in 1927. The identification in that case was made by Dr. Cram from specimens forwarded to the Zoological Division from Porto Rico. It was reported from the United States by Canavan in 1929. In Canavan's cases, however, the hosts, *Acryllium vulturina* and *Caccabis saxatilis chukar,* originated in Africa and Asia respectively. In 1926 this nematode was found in the small intestine of two local guinea hens at Jeanerette, Louisiana. This is, therefore, the first report of the occurrence of this nematode in native birds in the United States. The only serious discrepancy that I have been able to find in the existing descriptions is, that Leiper reports the length of the spicules as 0.9 mm. whereas all the other reports give the length of the spicules as approximating 3 mm. Both in the Porto Rico and in the Louisiana specimens the spicules are about 3 mm. in length. In all other respects they agree with the existing descriptions.

Dr. M. C. Hall discussed the influences responsible for the growing importance of parasitism in wild life and related his observations concerning the infestation of Rocky Mountain sheep with lung-worms and infestation of trout in lakes of the Yellowstone National Park with the plerocercoid of *Dibothrium cordiceps.* Doctor Hall also gave an account of his experiences in the capture and treatment of pelicans for the adult tapeworms.

Dr. E. W. Price presented the following note on Macrobilharzia Travassos.—In my paper, A Synopsis of the Trematode Family Schistosomidae, etc., the genus Macrobilharzia Travassos, 1923, was dropped as a synonym of Ornithobilharzia Odhner, 1912, because the characters given for the male (the female being unknown at the time) were so similar to those of Ornithobilharzia that the separation of the two genera appeared unwarranted. In the same paper a new genus, Paraschistosomatium, was proposed for what appeared to be a new species, *P. anhingae.* This species was based upon a single female. Recently the writer had an opportunity of examining a number of specimens of schistosomes, both male and female specimens, collected by Mr. Allen McIntosh from *Anhinga anhinga* in Florida during the spring of 1930. As a result of this examination the writer is convinced that the genus Macrobilharzia should be revived as a valid genus and Paraschistosomatium suppressed as a synonym. On the basis of a study of this additional material the following diagnosis of the genus is given:

*Macrobilharzia* Travassos, 1923.

*Synonym.—Paraschistosomatium* Price, 1929.

*Generic diagnosis.*—Schistosominae: Female shorter and much smaller than male. Male with well developed gynaecophoric canal. Suckers present. Cuticula

spiny. Digestive tract similar to that in Schistosoma. Testes numerous, up to 250, commencing a short distance caudad of acetabulum and extending into posterior half of body. Genital pore immediately caudad of acetabulum. Female slender, flattened, and tapering toward the extremities. Cuticula smooth. Oral sucker subterminal, well developed; acetabulum pedunculated. Esophagus simple; intestinal branches with short median and lateral diverticula; common cecum short. Ovary spirally curved, situated in posterior third of body. Seminal receptacle present. Vitellaria well developed and extend from a short distance caudad of acetabulum to posterior end of body; the follicles are situated along the intestinal branches cephalad of the level of the posterior pole of ovary, while caudad of this point they fill the intercecal space. Uterus long and filled with eggs.

Type species.—*Macrobilharzia macrobilharzia* Travassos, 1923.

Dr. G. Steiner reported on some infection and host transfer experiments with *Aphelenchus fragariae*. The experiments were made with the standard number of 30 adult specimens for each observation. The nemas were put on the various leaves, allowed to remain undisturbed and after a certain length of time (one to several days) the attempt was made to find and recover them. Of course in no instance were all recovered, but most often so many specimens were located that accurate conclusions as to their behavior could be drawn.

*Aphelenchus fragariae* transferred from strawberry buds to the under side of young leaves of Chrysanthemum would partly perish on the leaf surface or enter the leaf tissue, coil up and apparently stay in a dormant stage. This behavior seems to be similar to that of animal parasites which, after entering a "wrong" host, encyst themselves. *Aphelenchus fragariae* transferred from strawberry buds to young begonia leaves was always recovered in an active stage in the leaf-tissue, although a permanent and definite establishment of this form in begonia plants under normal conditions has not been seen. Studied on the strawberry plant itself, *Aphelenchus fragariae* seems to prefer to move on the surface of the plant but also enters the tissue of leaves, stems and runners and is able to travel inside the plant.

The one hundred thirty-second meeting was a dinner on October 18, 1930.

The one hundred thirty-third meeting was held at the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland, November 15, 1930.

The following note by E. E. Wehr was read: Occurrence of *Desmidocercella numidica* in herons in the United States.—In 1916, Skrjabin created a new genus, Desmidocerca, to include a very interesting and, in many ways, unique species of nematode, *D. aerophila,* which had been collected from the air sacs of *Ardea cinerea* in Russian Turkestan. Later, in 1920, Seurat described a new species, *D. numidica,* from Algeria, which he placed in the genus created by Skrjabin in 1916; up to the present, his finding is the only one on record for this species. These species are of special interest because there are carried on into adult life distinctive characters that really belong to the larval stage, and which are here maintained throughout the life of the adult individual. It was because of this unique feature that Cram, in 1927, created a new family, Desmidocercidae, for the reception of this genus. It is quite evident from a comparison of the description of the two above-mentioned species that they present very distinct differences. The more important ones are: In both the male and female of *D. aerophila,* the tail is provided with a terminal clump of filiform papillae; this clump is absent in *D. numidica,* but the tail of the female has two small knobs on each side. The esophagus of the former species extends for a distance approximately two thirds the length of the worm; that of the latter species extends only about one eighth to one ninth of the body length. In *D. aerophila,* the vulva is found in the posterior half of the body; in *D. numidica* it is in the anterior half near the union of the esophagus with the intestine. It was due, chiefly, to the difference in the position of the vulva of the two species that Yorke and Maplestone, in 1926, created a new genus, Desmidocercella, for the reception of Seurat's species, *D. numidica.*

On August 29, 1930, specimens of *Desmidocercella numidica* were found by the writer in a heron at Miles City, Montana; they were in a more or less coiled condition on the inner surface of the reflected portions of the peritoneum separating the two groups of air sacs of the abdominal region. The worms are small, the males measuring 5 to 6 mm. and the females 6 to 7 mm. long, and are about the size of No. 50 sewing thread. The most unusual characteristic of this species of nematode is that no mature eggs have been found in the adult female. Nothing is known concerning the life history of this nematode. On one other occasion the author has examined specimens of *D. numidica* collected from a heron killed at Urbana, Illinois.

Dr. E. B. Cram gave an account of the International Veterinary Congress and the World's Poultry Congress held in London. She also reported the organization, through the efforts of Professor Skrjabin, of an international society of helminthologists.

George D. Daniel presented a note on the respiratory quotient of *Balantidium coli* from the pig, the R Q of the parasite indicating that the starch present in the environment is not the chief food substance of the organism. The R Q equals 0.8.

Dr. Robert Hegner discussed the problem of the absence of opalinid infusoria from the rectum of adult green frogs. Ten adult green frogs from Mount Desert Island, Maine, were found to be free from opalinids. All of many young green frog tadpoles were infected. Opalinids are lost during the metamorphosis of green frog tadpoles between the age when two legs are present and that when four legs are acquired. Young adult green frogs could not be infected *per os* or *per rectum* with opalinid trophozoites from green frog tadpoles. Opalinids were found in the tadpoles of the tree frog during all stages of metamorphosis and also in young adult tree frogs. Apparently some digestive secretion peculiar to the green frog appears during metamorphosis that renders the rectum of this species unfit as a habitat for opalinids.

Dr. N. A. Cobb discussed the spineret of nemas and exhibited a very ingenious mechanical device, invented by himself, to demonstrate the workings of the spineret valve. A second note by Doctor Cobb dealt with a very asymmetrical nema, *Bunonema inequale* n. sp., in which the right-hand cuticula is markedly developed, a characteristic which is constant for all species of this genus.

The following note by Dr. Albert Hassall was read: *Ixodes hirsti,* new name for *I. victoriensis.*—Hirst (1930; 575) proposed the name *Ixodes victoriensis* for a new species of tick. As this name is a homonym of *I. victoriensis* Nuttall, 1916, a letter was sent to Dr. Hirst on May 21, 1930, inviting his attention to this fact and suggesting that he propose a new name. Subsequently it was learned that Dr. Hirst had died at sea on May 4. Accordingly the name *Ixodes hirsti* is proposed for *I. victoriensis* Hirst, 1930, not Nuttall, 1916.

Dr. G. F. Otto presented notes on (1) the passage of ascaris eggs through animals. The question as to the possible rôle of scavenger animals in the epidemiology of ascariasis has been raised several times. Dogs and chickens are common in dooryards of most ascaris infested homes in southern United States and chicken feces are found in the houses and yards. Human ascaris and trichuris eggs in apparently viable condition were recovered from many of these droppings. To determine the fate of all eggs ingested, eggs were taken from the uteri of pig ascaris, shaken in water, counted and calculated amounts fed by capsule or pipette to chickens, dogs and a cat. Feces were then collected until after two stools were negative by Lane's D C F method. Results: 325,000; 275,500 and 439,500 eggs were fed to and 76.2 per cent, 52.3 per cent and 72.8 per cent recovered from three dogs; 327,500 eggs fed to and 69.0 per cent recovered from a cat; 203,000, 326,000 and 305,000 eggs were fed to and 16.5 per cent, 40.5 per cent and 33.4 per cent recovered from three chickens. All eggs recovered appeared viable and became embryonated when cultured on sand or in water. In chickens alone was there any evidence of destruction; many egg shells, in addition to the percentage listed, were found apparently crushed by the grinding action of the gizzard. The loss

of eggs in the mammals may have been due to digestion, though no direct evidence was seen; or more likely to the loss of some eggs clinging to the feeding pipette though every precaution was taken against this. These results indicate that chickens at least *may* act to destroy eggs and on the other hand *may* carry some eggs to potentially infective points. In southern United States, however, it seems unlikely that animals are important in either way in the epidemiology of ascariasis. This latter point will be discussed soon in the *American Journal of Hygiene* (Otto, Cort and Keller).

(2) Massive acquisition of ascaris by young child.—A male child 22 months old, giving history of "spontaneous" passage of ascaris six or seven months previously, was given home medication because not treated with the other children. All the worms, 174, resulting from the first four passages were saved and presented by the mother as proof of inability to diagnose the case. All worms were immature, ranging from 6 to 10 inches long and very thin, all apparently being of the same or nearly the same age, representing probably 3 to 5 weeks' development. Three of the females were approaching maturity and contained eggs in the uteri. This very graphically shows the massive infections which are acquired and serves to support the view that there is not the slow building up of infections with ascaris but rather a rapid turnover resulting from the massive acquisition and loss of worms. It further supports the view that those individuals in a worm-infested area negative by a given examination are not the negative element of the population but only those who happen to be negative at that time.

D. A. Shorb reported on the experimental infestation of white rats with *Hepaticola hepatica*.—Of seventy-one wild rats obtained in and around Baltimore, 47.9 per cent were infected with *H. hepatica*. Six white rats fed on the infected livers of these wild rats did not develop the infestation, but the eggs passed through the intestine unchanged. These eggs were recovered from the feces and became embryonated in twenty-five to forty-two days at 30°C. Fifteen white rats became infected when fed on these embryonated eggs, while eggs in pieces of the rats' livers but not passed through an animal were developed only to the early morula stage in forty-two days. Eggs from infected livers fed to a cat also developed vermiform embryos in thirty days.

Dr. M. C. Hall extended his remarks at a previous meeting concerning the importance of parasitism in wild life and reported the formation of a committee with representatives from various government departments to study the problem.

A note was read for Dr. C. W. Rees: The treatment of notoedric mange in rabbits.—About a year ago a colony of a dozen rabbits at this laboratory was attacked with body mange, and *Notoedres minor cuniculi* was demonstrated on several occasions in the scrapings of the scabs. Only occasional attempts were made to treat the animals and all but three succumbed. Early in January, 1930, the writer decided to experiment with kerosene. To prevent the loss of the hair this liquid was diluted with an equal volume of cottonseed oil and to the mixture 5 per cent of liquid phenol (prepared by melting crystals) or 10 per cent of lysol was added with the expectation that healing of the many open sores would thus be promoted. The remedy was applied by means of a small tin sprayer such as is commonly used in house-fly control. When the treatments were commenced the disease had spread, in all three rabbits, over the face, ears, neck, and all four feet, resulting in almost complete loss of hair over the parts affected and the replacement of the latter by scabs and open sores. Up to the present time, April 1, 1930, three treatments have been applied to each rabbit and all appear to be completely cured. The hair has grown normally and no traces of scabs can be found. The treatment of each rabbit required the time of two people for only a few seconds. Since the common practice is to kill mangy rabbits because of the tediousness, uncertainty, and expense of treatment, the above method deserves test on a more extensive scale.

O. R. Causey presented a note on a medium for growing blow-fly maggots.

Miss M. F. Jones presented the following notes: (1) On the loss of an experimentally produced infestation of tapeworms in a chicken.—A chicken was fed

cysticercoids of *Raillietina cesticillus* on March 20 and by April 15 the bird was passing gravid segments of that tapeworm. Segments for experimental work were collected on various dates up to and including June 24. Fecal examinations on July 1, 15 and 23 were negative for tapeworm segments. At postmortem examination on August 22, no parasites were found. Thus an infestation which was present for at least three months was lost by the end of approximately five months.

(2) On the life histories of species of Raillietina.—A specimen of the ground beetle *Amara* (*Amara*) sp. collected at a poultry farm near Beltsville, Maryland, was found to be naturally infested with numerous cysticercoids identified as *Raillietina* (*Paroniella*) *magninumida,* a cestode of guinea fowl. Previously, cysticercoids of this species had been obtained but only as artificial infestations in another ground beetle, *Selenophorus pedicularius.* The material from *Amara* (*Amara*) sp. was fed to two guinea fowl chicks and to one turkey poult. Upon postmortem examination, after approximately three weeks, one guinea fowl chick was found to be infested with three mature specimens of *R.* (*P*) *magninumida,* while the other two birds were free from parasites. Five guinea fowl chicks held as controls were free of all helminths.

Cysticercoids of the fowl cestode *Raillietina cesticillus* were developed in the ground beetle *Amara* (*Amara*) sp.; over 100 cysticercoids were present in one beetle. The material was fed to two turkeys and to one guinea fowl chick. Numerous specimens of *R. cesticillus* were obtained from the guinea fowl chick upon postmortem examination, and one specimen from one of the turkeys. The other turkey remained free from parasites as did all control birds. The beetle had been given segments of *R. cesticillus* from chickens, but since it had been collected at a poultry farm where chickens, turkeys and guinea fowl are kept, the possibility of an original natural infestation is recognized.

C. M. Johnson reported on trypanosome infections in experimental animals.—Experimental trypanosome infections in laboratory-bred rats has disclosed the fact that the animals die of asphyxia. The trypanosomes in one manner or another, at present under investigation, bring about an intravascular obstruction of the circulation. This is clearly shown by the pronounced pulmonary edema and intracardial and intravascular blood clots revealed at autopsy. The two theories concerning the cause of death that workers have held previously, i. e., uncompensated acidosis and hypoglycemia, seem to this author to be the result of the condition described above and not the primary cause of death.

R. M. Stabler presented a note on the variations in a single race of coli-like amoeba.—A case of infection with a coli-like amoeba has been followed since November 23, 1929. During this period, 293 examinations have been made and since May 8, 1930, an examination has been made of every stool passed by the host. A study of the living material and the fixed and stained preparations reveals the fact that this strain of amoeba possesses all the morphological and cytological characteristics attributed to *Endamoeba coli* and all those attributed to *Councilmania lafleuri,* with the exception of the fact that never once, despite the thousands of cysts that have been seen, has the process of budding and the escape of uninucleate amoebulae been observed in the fresh saline preparations. Individual trophozoites have repeatedly been observed to pass from a state producing clear, explosive pseudopodia to one in which the amoebae moved rapidly and with the production of granular pseudopodia, showing absolutely no clear ectoplasm. The other morphological features, such as the karyosome, chromatoids, etc., show a similar inclusion of the types of both *coli and Councilmania.* It is felt that a single race of a single species of amoeba was being dealt with, for the organism always showed the same variations and these were always of the same ratio, never more or less of the *coli* type at any one time than another. This is not what might be expected if there was a mixed infection. It is concluded that the coli-like amoeba in question is of a single species and race, showing all the features attributed to both *Endamoeba coli* and *Councilmania lafleuri.* The only other organism present in the case was *Blastocystis hominis.*

Frederic Fish presented a progress report of work dealing with the destruction of oocysts of *Eimeria tenella* Railliet and Lucet, 1891.—The time necessary to produce 100 per cent mortality of unsegmented oocysts by heat is inversely proportional to the degree of heat used. At 45°C, all succumb within 24 hours; 50°C, 1½ hours; 55°C, 3 minutes; 60°C, 15 seconds; 70°C, 15 seconds; 80°C, 5 seconds; 90°C, 5 seconds. Segmented oocysts are equally susceptible to heat as unsegmented ones in a ten minute exposure. Both are killed at approximately 54°C in ten minutes. The comparative killing power of certain reagents against the washed, unsegmented oocysts of this species was found to be: Iodine Suspensoid, Merck, less than one hour; Cresol 2 per cent, 4 to 8 hours; Cresol 5 per cent, 4 to 8 hours; Phenol 2 per cent, slightly over 48 hours. The unsegmented oocysts of this species are killed by exposure to one zinc sulfide unit of ultra-violet rays.

Dr. Rudolph Wetzel presented notes, as follows:

(1) On the differentiation of the third stage larvae of *Strongylus equinus, S. edentatus* and *S. vulgaris:* The ensheathed third stage larvae of *S. equinus, S. edentatus* and *S. vulgaris* are distinguished by a long whip-like tail which occupies about one-third of the whole body length. The larvae can be separated from one another by (a) The general form. While the absolute length of the larvae of each species varies considerably according to the conditions under which they are raised, it can be said that as a rule, the larvae of *S. vulgaris* are the longest and have the largest diameter. The larvae of *S. equinus* are somewhat shorter and have a narrower diameter, thus appearing more slender than the former. The larvae of *S. edentatus* are about intermediate between the above species. (b) The relative length of the esophagus. The following data are based on 75 to 90 measurements of each species. The measurements indicate that the esophagus occupies in the larvae of *S. equinus* 32.5 per cent, in the larvae of *S. edentatus* 28.2 per cent and in the larvae of *S. vulgaris* 20.1 per cent of the body length. (c) The number and shape of the intestinal cells. The larvae of *S. equinus* have 8 dorsal and 8 ventral rather long triangular cells. In the larvae of *S. edentatus* the intestinal cells number 20, while in the larvae of *S. vulgaris* the intestinal tube is formed by 32 rather short triangular cells. (d) The shape of the tail end. The more or less blunt tail end of *S. equinus* larvae is supplied with a small trilobed appendix. The larvae of *S. edentatus* have a rounded tail end without an appendix, while the tail is more pointed in the larvae of *S. vulgaris.* There are further differences in the finer structure of the larvae such as in the arrangement of the esophageal glands.

(2) Among some nematodes from the gizzard of a mallard (*Anas platyrhynchos*) forwarded to the Zoological Division by E. E. Wehr, Miles City, Mont., there were found some specimens of *Amidostomum cheevreuxi* Seurat 1918, which is probably identical with *A. skrjabini* Boulenger, 1926. The worm has until now only been reported from Africa from *Himantopus himantopus* and *Anser albifrons.*

B. G. Chitwood reported on the occurrence of flagellate spermatozoa in a nematode, *Trilobus longus.*

The one hundred thirty-fourth meeting was held December 20, 1930.

Dr. C. W. Stiles related his experiences as a delegate to the International Exhibit of Hygiene at Dresden and the International Zoological Congress at Padua. He exhibited a specimen of the pork responsible for the epidemic of trichinosis in Plauen in 1860, the outbreak which led to Zenker's brilliant work in discovering the pathology of *Trichinella spiralis.*

A communication from Dr. N. R. Stoll was presented correcting his note given at a previous meeting (Jour. Parasit., 17: 54), to read as follows: Experimental work indicates that the goat can be infected with the sheep strain of *Haemonchus contortus.* It has long been known that the same species appears in both hosts. This demonstrates experimentally that a strain of such species from the sheep will infect the goat.

Dr. Wm. A. Hoffman reported on the removal of two specimens of *Syngamus laryngeus* from the posterior pharynx of a male Porto Rican, the third case on record from man. Dr. Hoffman also presented a note on allergic reactions to Schistosome infestation, a typical reaction having been secured in a boy whose symptoms disappeared after treatment for *Schistosoma mansoni*.

Dr. H. A. Kreis reported on some infection experiments made with *Tylenchus dipsaci* on sweet and Irish potatoes. A first group of experiments was performed to determine if the green parts of sweet potatoes were, like the tubers, attacked by *Tylenchus dipsaci*, and, if so, what the symptoms were. Infections of leaves and stems were unsuccessful; it was, however, possible to observe the active sucking of the nemas on the youngest parts of a bud whereupon the damaged tissue turned black.

In a second group of experiments, various possible ways of infestation from sweet potato tuber to sweet potato tuber were tried: 1. A cylindrical piece of diseased tuber was inserted into a similar opening cut in a healthy tuber. Result: the disease was transferred. 2. The half of a healthy tuber was placed cut to cut on a diseased tuber with the result that the healthy tuber became diseased. 3. A healthy and a diseased tuber were placed side by side with moistened surfaces. The disease transferred. 4. A piece of the peeling of a diseased sweet potato tuber was placed on a healthy tuber—a transfer that also proved successful. From these experiments it may be concluded that the *Tylenchus dipsaci* disease of sweet potatoes is transferred from tuber to tuber. It is thought that this may especially happen in the storage bins, where the tubers are in close contact under a temperature that is suitable to Tylenchus.

Infestation experiments in the fields have not yet been made to an extent permitting definite conclusions. The Irish potato is known to be attacked by this pest in several foreign countries; the disease is, however, not yet recorded from the United States. A series of transfer experiments of *Tylenchus dipsaci* from the sweet potato to the Irish potato was therefore made, but up to the present without positive results.

Dr. E. M. Nighbert presented the following notes: (1) Two males of *Ascaris suum* in copula with one female.—During 1929, the writer and his assistant, Mr. J. W. Connelly, making a postmortem examination of a pig heavily infested with ascarids, observed two male ascarids in copulation with a single female. The coupling was complete and the three worms were lifted out, cleansed and placed in preservative for keeping in our collection. The specimens remained intact for two or three days before the worms separated as the preserving fluid caused contraction of the body tissues. Most of the reports of two males simultaneously in copula with one female appear to deal with strongyles.

(2) Unusual location for adult kidney worm (*Stephanurus dentatus*) of swine.—Last year during a post-mortem examination of a pig, an adult male and female kidney worm were found located under the gastric serosa inferior to the lesser curvature of the stomach. The uterus of the female was filled with eggs. Ordinarily this parasite reaches maturity only in the perirenal region.

(3) Swine sanitation as a control measure for the thornhead worm (*Macracanthorhynchus hirudinaceus*) of swine.—The use of the sanitation system of raising pigs appears to be effective in the control of thornheads. In the years 1926 to 1928 inclusive, 44 pigs raised on a farm badly infested with thornheads were examined postmortem at time of marketing; 40 of the pigs showed from 18 to 257 thornhead worms per pig. Only 4 of the pigs were free of this parasite. In the year 1929 this farmer raised his pigs under the sanitation system, namely, in cultivated fields away from the contaminated premises. Sixteen of the pigs were examined postmortem at time of marketing; 6 of the pigs showed thornhead infestation with from 3 to 20 worms per pig; 10 pigs were free of the parasite. With no control measures for three years 90.9 per cent of the pigs were infested and 9 per cent not infested. The incidence of thornheads was cut to about 40 per cent of the original infestation, and the degree of infestation measured in number of worms per pig was greatly reduced.

W. H. Wright, *Secretary*

# BOOK REVIEWS

Handbook of Protozoology. By Richard R. Kudo, University of Illinois. 451 pages, 175 figs. Charles C. Thomas, Springfield, Ill.

The book opens with a striking citation from Leidy and holds the interest of the student even through the purely taxonomic portions. The first three chapters cover the history of the field and the morphology, physiology and reproduction of protozoa. The author has chosen to handle this phase of the subject briefly because of the effective treatment it has received at other hands. The remaining thirty chapters of the book are devoted to the taxonomy and biology of the common protozoa. The abundant use of keys and the incorporation in each chapter of important recent literature on the organisms treated therein will appeal strongly to all students, even though the references to literature are few in number and are intended merely as guides to the already greatly extended literature on protozoa. The descriptions of individual genera are drawn up briefly with great care and almost every one of the species mentioned has been illustrated. Special praise should be given to the figures which are not only abundant and new but unusually well drawn and reproduced. The book is splendidly printed and the publisher deserves congratulations for the effective manner in which he has presented the good work of the author both in text and in illustrations. The number and variety of known forms among the protozoa is already very large and to bring the material within reasonable compass calls for severe pruning. To serve the needs of students who are not specialists in this field the selection must be made with care in order to secure a well balanced picture of the group. Professor Kudo has succeeded in his task. The work is not only well done; the book is unique and will give to students and others interested in the field a summary of the various types of protozoa which they will find most useful.

Studies in the Parasitology of Malaria. By Lieut.-Colonel R. Knowles, R. Senior White, and Assistant Surgeon B. M. Das Gupta. (Calcutta School of Tropical Medicine and Hygiene.) 436 pages, 59 charts, 27 tables and 10 maps. Thacker, Spink & Co., Calcutta.

This important study of the factors that influence a laboratory diagnosis of malaria is based upon extensive records accumulated at the Calcutta School of Tropical Medicine and on the Bengal-Nagpur Railway. The authors have considered first the regional origin of the patient with special reference to the distribution of the species of Plasmodium. Certain localities in other regions and continents are discussed and a very complete study made of India. This extensive survey is fortified by maps and distribution tables of frequency and a mass of important and well analyzed data. The factors underlying the distribution of Plasmodium, the seasonal distribution of the disease and the various conditions of age, race, sex and occupation of the patient discussed. After a consideration of treatment and technique the authors comment on the important problems demanding investigation in this field pointing out the large gaps in the knowledge of geographical distribution of species and the bionomics of the malarial parasites. A brief summary and an extended but confessedly incomplete bibliography closes the monograph.

The Fauna of British India. Cestodes, Vol. II. By T. Southwell (School of Tropical Medicine, Liverpool). 262 pages, 133 figs. Taylor & Francis, London.

This is the second part of a study, the first part of which was reviewed in the Journal for September, 1930. In this part is included material on the following families: Taeniidae, Anoplocephalidae, Davaineidae, Hymenolepididae, Dilepididae

Mesocestoididae, Nematotaeniidae, Amabiliidae, Diploposthidae, Acoleidae, Tetrabothriidae, Dioecocestidae. At the close the author considers the series of genera of uncertain systematic position or identity. The plan followed in the text is that already discussed in the review of the first volume. The author's thoroughness and accuracy coupled with an interesting style makes the book more attractive than the ordinary systematic study. Not only those interested in the region covered but any who are working on these parasites will find this a valuable contribution.

Les Moustiques de la Cochinchine et du Sud-Annam. By B. E. Borel (Pasteur Institute of Indo-China at Saigon). 423 pages, 128 text figures, 7 tables. Masson et Cie, Paris, France.

This publication, which is a contribution from the Pasteur Institute of Indo-China at Saigon, is one of those precise studies essential for the successful handling of the great plagues of the tropics, especially in this case malaria, in a particular region. In the preface Professor Roubaud of Paris relates the training of the author, his personal fitness for the work, the inception of his duties in 1894 as director of the newly created laboratory at Saigon for the study of antimalarial measures, his ardent devotion to the task of mastering the systematic and biological characters of the mosquitoes in his territory. Out of his activities grew a veritable monograph of the Mosquitoes in Cochinchina and South Annam. On the untimely death of Borel in 1928 he left his work so far advanced that it was possible with the aid of friends to issue it in the form of a special monograph. The monograph is preponderatingly taxonomic but includes also material of biological interest and will be of great value to workers in the field.

Die Tierischen Parasiten und Einige Parasitäre Krankheiten des Menschen in Tadshikistan. By Dr. E. N. Pawlowsky. 208 pages, 16 tables, 48 text figs. Leningrad Druckerei der Akademie der Wissenschaften U. S. S. R. (Russian with German summaries).

Russian scientists are contributing richly to the literature of parasitology and extending knowledge of the distribution of parasites in regions hitherto almost unknown. The work issued by Professor Pawlowsky under the title given above includes 17 separate contributions by 9 different authors; of those 9 articles are from his own pen. In the summer of 1928 a parasitologic expedition of the Zoological Museum of the Academy of Sciences was sent out to investigate poisonous animals, parasites and the vectors of infective diseases in Central Asia. Of the studies here reported two each deal with malaria, mosquitoes, bot flies and poisonous insects, and three each with ticks and human helminthology; others report on elephantiasis, etc. Further publications on the work of this expedition are promised and will be awaited with interest.

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The actual dates of the issue of Volume XVII of the Journal were as follows:

No. 1, October 4, 1930
No. 2, December 6, 1930
No. 3, April 15, 1931
No. 4, June 19, 1931